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Cardiorespiratory control in the perioperative patient: from bench to bedside

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C a r d i o
R e s p i r a t o r y
C o n t r o l
i n t h e
P e r i o p e r a t i v e
P a t i e n t

from bench to bedside

Diederik Nieuwenhuijs

CardioRespiratory Control in the Perioperative Patient

from bench to bedside

PROEFSCHRIFT

ter verkrijging van de graad van Doctor
aan de Universiteit Leiden,
op gezag van de Rector Magnificus Dr. D.D. Breimer,
hoogleraar in de faculteit der Wiskunde en
Natuurwetenschappen en die der Geneeskunde,
volgens besluit van het College voor Promoties
te verdedigen op woensdag 4 september 2002
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Diederik Jan Friso Nieuwenhuijs
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Overige leden: Prof. Dr. J.G. Bovill
 Prof. Dr. C.J. Kalkman (UMCU)
 Dr. E.Y. Sarton

Side effects of anesthesia include nausea and vomiting, but it is respiratory depression that is potentially life-threatening.

Scientific American, February 2002

Aan mijn ouders

The investigations described in chapters 2-8 of this thesis were performed in the Laboratory of Physiology, Leiden University Medical Center, under the supervision of Dr. A. Dahan and Dr. L. Teppema, those described in chapter 9 in the Royal Infirmary of the University of Edinburgh under the supervision of Dr. G.B. Drummond and Dr. P.W. Warren. All studies in this thesis were supported by Grant MW 902-19-144 from The Netherlands Organization for Pure Research (ZorgOnderzoek Nederland Medische Wetenschappen-NWO, The Hague, The Netherlands).

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1 Introduction

ANESTHESIA has profound effects on the respiratory control system. It has long been known that anesthesia may diminish pulmonary ventilation, and hypercapnia is commonplace if spontaneous breathing is preserved. Studies looking at the incidence of postoperative respiratory complications show that hypoxemia is a common problem at the emergence of anesthesia in the postanesthesia care unit (PACU).^{76,90} During recovery from anesthesia, hypoxia, hypercapnia, and acidosis have several causes: residual anesthetic and analgesic drugs at their effect site, atelectasis, reduced cardiac output, upper airway obstruction, analgesic/sedative medication, pain/stress, and underlying disease. The patient may continue to breathe during a hypoxemic episode, but hypoxia and hypercapnia have further effects. They cause sympathetic nervous system activity, which can lead to tachycardia, hypertension and ischemic ECG changes. Afferent input from the peripheral chemoreceptor is an important stimulus to arousal, the clearing of upper airway obstruction and the subsequent hyperventilatory response to correct any hypoxia, hypercapnia and acidosis. Therefore it is of utmost importance to understand the effect of anesthetics and analgesics on cardiorespiratory control and the mechanism of action of these agents.

Control of Breathing

Breathing results from activity of the respiratory centers in the brainstem and is well adjusted to the metabolic and non-metabolic needs of the body. Optimal adjustments are possible by incorporating information from various sites in the body. With respect to the metabolic control of breathing, the chemical composition of arterial blood primarily regulates breathing through effects on the peripheral and central chemoreceptors. The peripheral chemoreceptors in the carotid bodies are sensitive to changes in arterial pH, PCO_2 and PO_2 . The central chemoreceptors on the surface of the ventral medulla are sensitive to changes in brain tissue PCO_2 and pH. To maintain a chemical equilibrium in the body, the metabolic ventilatory control system makes use of two reflex pathways. The peripheral chemoreflex loop consists of the peripheral chemoreceptors, the sinus nerve, sites in the brain stem that receive and process afferent input from the carotid bodies, the brainstem respiratory centers and the neuromechanical link between brainstem and ventilation (phrenic nerve, spinal motorneurons, diaphragm, intercostal nerves and muscles, lungs). The central chemoreflex loop involves the central chemoreceptors, and neuronal connection between these receptors and the brainstem respiratory centers and the above mentioned link between respiratory centers and ventilation (*i.e.*, the pathway common to both chemoreflex loops).^{37,169,196}

Pure chemical control of breathing operates only during non-rapid-eye-movement (non-REM) sleep and anesthesia (in spontaneous breathing patients). During wakeful-

ness and REM-sleep, another equally important system, the behavioral control system, will influence breathing and may even temporarily override the chemical system. Behavioral control of breathing allows for adjustment of breathing to specific situations such as speech, singing, reading, eating, diving, *et cetera*.²¹¹ In the postoperative patient various other systems will influence breathing, such as the pain-related control of ventilation and the stress response to surgical stimulation. Clinical and experimental studies show that pain and surgical stimulation act as a chemoreflex-independent respiratory stimulant in the awake, sedated and anesthetized states.^{109,163,173}

The aim of this thesis is to increase our insight in the cardiorespiratory control of perioperative patients. Studies were performed in animals, volunteers and patients. They were designed to answer the following questions:

1. What is the role of the carotid body in the control of breathing in man?
 2. What is the mechanism of anesthesia-induced depression of the peripheral chemoreflex loop and are we able to develop cheap and effective regimens to prevent depression of this vital chemoreflex?
 3. How do intravenous and inhalational anesthetics and opioids, given alone and in combination, affect cardiorespiratory control?
 4. Is the depression of anesthetics and analgesics on respiration, counterbalanced by the stimulatory effects of pain and stress?
- In *Chapters 2 and 3*, items 1 and 2 are addressed. In *Chapter 2* respiratory studies were performed in healthy volunteers as well as in unilateral and bilateral carotid body resected patients in order to quantify the influence of the carotid bodies on the control of breathing. Studies performed are multiple steps into and out of hypercapnia according to a multifrequency binary sequence (MFBS) recently developed in Oxford to optimize the study of the peripheral chemoreflex loop.¹⁴⁴
 - In *Chapter 3* hypoxic studies were performed in healthy volunteers in the absence and presence of antioxidants (iv ascorbic acid and oral α -tocopherol) during the inhalation of the potent volatile anesthetic halothane. Halothane, at already sub-anesthetic concentrations (0.05–0.1 end-tidal %) causes profound depression of the carotid bodies and consequently of the ventilatory response to hypoxia.⁴⁷ This protocol was developed to test the ability of antioxidants to prevent halothane-induced depression of the hypoxic ventilatory response. The administration of antioxidants makes sense taking into account the vast literature showing the involvement of free radical species in oxygen sensing at the carotid bodies, and the production of radicals species from halothane due to its reductive metabolism.^{95,96}

- In *Chapters 4 and 5*, the influence of the intravenous anesthetic propofol on cardiorespiratory control is discussed. The results of experiments on various cardiorespiratory and EEG parameters such as the acute and sustained hypoxic ventilatory response, dynamic carbon dioxide ventilatory response (MFBS), heart rate and bispectral index of the EEG are reported. Furthermore, the possible site of action of propofol within the chemical ventilatory control system is discussed (item 3).
- In *Chapters 6 and 7*, the effect of combining opioids and anesthetics on the cardiorespiratory control system is described. The nature and magnitude of interaction of an anesthetic-opioid combination on resting ventilation, resting end-tidal carbon dioxide concentration, blood pressure, heart rate and bispectral index of the EEG and the steady-state ventilatory responses to carbon dioxide and acute hypoxia is assessed using the technique of response surface modeling (item 3).
- In *Chapter 8*, the influence of tramadol on ventilatory control in the anesthetized cat is discussed. To examine the involvement of the μ -opioid receptor in tramadol effects on respiration, the ability of naloxone, an opioid-antagonist, to reverse the respiratory effects of tramadol was studied (item 3).
- Finally, in *Chapter 9*, the complex of factors that interact on the cardiorespiratory control system in postoperative patients is examined. Respiratory studies are performed in patients shortly after major abdominal surgery as well as weeks to months later so that these subjects could serve as their own control. Breathing was tested by applying ramp-like increases in end-tidal PCO_2 combined with concomitant ramp-like decreases in end-tidal PO_2 . This stimulus was chosen to mimic the changes in arterial gas composition that occur during upper airway obstruction (item 4).

SECTION 1

Physiology

2 Modeling the ventilatory response to carbon dioxide in humans after bilateral and unilateral carotid body resection (CBR)

IT IS AXIOMATIC that the respiratory chemoreceptors sense and respond to changes in the composition of their immediate microenvironment.⁷⁸ In humans, the ventilatory response to a step change in end-tidal CO_2 yields a fast ($\tau \sim 10$ s) and a slow component ($\tau \sim 120$ s).^{12,40} Two sets of chemoreceptors are thought to elicit these two components: the peripheral chemoreceptors, causing the fast component and located in the carotid bodies at the bifurcation of the common carotid artery, and the central chemoreceptors, causing the slow component and located in the ventral medulla.^{12,40,55} Validation of the (carotid body)-origin of the fast component in humans is a difficult task and has not been accomplished satisfactorily as yet. Studies in animals,⁵⁵ and patients who have had bilateral carotid body resection (CBR) for the relief of asthmatic symptoms,^{12,91} or bilateral carotid endarterectomy for transient cerebral ischemia,²⁰⁸ suggest that the fast component of the ventilatory response to CO_2 arises from carotid body activity. However, it is questionable whether animal studies apply directly to humans, and in case of patients with underlying disease of the vessels and lungs, it is also possible that the effect on the \dot{V}_I-CO_2 response was related to any underlying process.

In this study, we sought to examine the ventilatory response to CO_2 of adult human subjects who had undergone bilateral and unilateral carotid body resection for carotid body tumors. Testing in patients with carotid body tumors prior to resection had revealed that the carotid body function had not been altered by the tumor formation. Furthermore, all of the tested subjects were otherwise healthy with normal lung and cardiovascular function.

METHODS

Patients and Volunteers

We recruited 14 patients and 7 volunteers after approval of the protocol was obtained from the LUMC ethics committee. Patients had undergone unilateral or bilateral resection of the carotid body 1 (2000) to 26 (1976) years before testing but were otherwise healthy. These patients had developed tumors of one or two carotid bodies (*glomus tumor or chemodectoma*), most of them due to a mutation of the SDHD gene on chromosome 11q23, as part of the head and neck hereditary paraganglioma. SDHD (succinate-ubiquinone oxidoreductase subunit D) is a small part of cytochrome b588 of the mitochondrial respiratory chain complex II. In the majority of Dutch patients (all part of the Dutch founder families) a missense mutation that changes ASP⁹² \Rightarrow Tyr was found.^{93, 203} Control patients were healthy volunteers matched for age and sex (table 1).

Table 1. Patient and volunteer characteristics

	male/female	age (yrs)	age range	weight (kg)	height (cm)
bilateral CBR	4/3	46 ± 8	28-51	73 ± 10	174 ± 16
unilateral CBR	4/3	41 ± 10	30-56	78 ± 18	177 ± 13
control	4/3	48 ± 11	31-59	73 ± 11	175 ± 9

Values are mean ± SD.

Apparatus

The subjects were comfortably seated in a hospital bed and breathed through a face mask (Vital Signs, Totowa, NJ). The gas flows were measured with a pneumotachograph connected to a pressure transducer and electronically integrated to yield a volume signal. The volume signal was calibrated with a motor-driven piston pump (stroke volume 1 l, at a frequency of 20 min⁻¹). Corrections were made for the changes in gas viscosity due to changes in oxygen concentration of the inhaled gas mixtures. The pneumotachograph was connected to a T-piece. One arm of the T-piece received a gas mixture with a flow of 50 L/min from a gas mixing system, consisting of three mass flow controllers (Bronkhorst High Tech BV - F202, The Netherlands) with which the flow of O₂, N₂ and CO₂ could be set individually at a desired level. A Personal Computer provided control signals to the mass-flow controllers so that the composition of the inspired gas mixtures could be adjusted to force end-tidal oxygen and carbon dioxide concentrations ($P_{ET}O_2$ and $P_{ET}CO_2$) to follow a specified pattern in time, independent of the ventilatory response. The in- and expired O₂ and CO₂ concentrations and the arterial hemoglobin-O₂ saturation (S_pO_2) were measured with a Datex Multicap gas monitor (near the mouth) and Datex Satellite Plus pulse oximeter, respectively (Datex-Engstrom, Helsinki, Finland). The gas monitor was calibrated with gas mixtures of known concentration delivered by a gas-mixing pump (Wösthoff, Bochum, Germany). $P_{ET}O_2$, $P_{ET}CO_2$, tidal volume, respiratory frequency, inspired minute ventilation (\dot{V}_i) and S_pO_2 were collected and stored on disc for further analysis. The data steering and acquisition software was custom build (RESREG and ACQ) by Erik Kruyt and Erik Olofsen and displays the ventilation data on-line in real time.

Study Design

Each subjects rested for 30 min after arriving in the laboratory. Next, two hypercapnic studies were performed at the background of normoxia, followed by a 20-min hypoxic study ($P_{ET}O_2$ 7 kPa), and, finally two hypercapnic studies at the background of mild hypoxia ($P_{ET}O_2$ 10 kPa).

Hypercapnic Studies: The end-tidal PCO₂ was varied according to a multi-frequency binary sequence (MFBS) that involved 13 steps into and 13 steps out of fixed $P_{ET}CO_2$ levels (low and high CO₂: 2 mmHg and 12 mmHg above the subjects normal air breathing value for $P_{ET}CO_2$) altogether lasting 1408 s (23 min and 28 s).¹⁴⁴ See figure 1 of chapter 6 for a schematic diagram of the $P_{ET}CO_2$ input function.* The MFBS experiments were performed at a background of normoxia or moderate hypoxia (to cause a more potent stimulus to the peripheral chemoreceptors) The hypoxic CO₂ studies started 20 min after the initiation of hypoxia, which was done to allow time for hypoxic ventilatory decline to develop prior to investigating the response to CO₂ (cf. Chapter 4).

*See for the rationale of using MFBS rather than step CO₂ input functions Chapter 5.

Sustained Hypoxic Studies: The $P_{ET}O_2$ was forced as follows: (1) 10 min at 15 kPa, (2) a rapid decrease to 7 kPa, (3) 20 min at 7 kPa ($S_{p}O_2 \sim 87\%$), (4) a rapid increase to 10 kPa (after which the last two hypercapnic studies were performed).

Data Analysis

Hypercapnic Study: In order to determine whether both the fast and slow components could be identified in the ventilatory response to $P_{ET}CO_2$, both single and a two-compartment model were fitted to the data. Both models were based on that of Bellville *et al.*¹² and Dahan *et al.*⁴⁰ The two-compartment model, describing central and peripheral chemoreflex parameters is given by:

$$(1) \quad \tau_c \frac{d}{dt} \dot{V}_c(t) + \dot{V}_c(t) = G_c [P_{ET,CO_2}(t - T_c) - B_k]$$

$$(2) \quad \tau_p \frac{d}{dt} \dot{V}_p(t) + \dot{V}_p(t) = G_p [P_{ET,CO_2}(t - T_p) - B_k]$$

$\dot{V}_c(t)$ and $\dot{V}_p(t)$ are the outputs of the central and peripheral chemoreflex loops. $P_{ET}CO_2(t - T_c)$ is the stimulus to the central chemoreflex loop delayed by the central transport delay time, $P_{ET}CO_2(t - T_p)$ the input to the peripheral chemoreflex loop delayed by the peripheral transport delay time. The parameters G_c and τ_c are the CO_2 sensitivity and time constant of the central chemoreflex loop. The corresponding parameters of the peripheral chemoreflex loop are denoted by G_p and τ_p . B_k is the apneic threshold or extrapolated $P_{ET}CO_2$ of the steady-state \dot{V}_i - $P_{ET}CO_2$ response at zero \dot{V}_i .

The noise corrupting the data is modeled through an external parallel pathway (\dot{V}_n).¹¹⁴ In most experiments a drift in the ventilation was present. We therefore decided to include a drift term in our model ($C \cdot t$). The total ventilatory response is made up of the sum of the contributions of the central and peripheral chemoreflex loops, the external noise, the drift term and the measurement noise term ($W(t)$):⁴⁰

$$(3) \quad \dot{V}_i(t) = \dot{V}_c(t) + \dot{V}_p(t) + \dot{V}_n(t) + C \cdot t + W(t)$$

The two-compartment model reduces to the one-compartment model by fixing G_p and thus component \dot{V}_p to zero. This results in the simple model:

$$(4) \quad \dot{V}_i(t) = \dot{V}_c(t) + \dot{V}_n(t) + C \cdot t + W(t)$$

The estimation of the parameters of the one- and two-compartment model was performed with an one-step prediction error method.¹⁴⁴

Sustained Hypoxic Studies: Mean values of the breath-to-breath data were chosen over identical time segments. Period *A* is the 1-min period before the 15-min of hypoxia; Period *B* the 3rd min of hypoxia; Period *C* the 20th min of hypoxia. Differences in \dot{V}_i between Periods *A* and *B* were defined as the acute hypoxic response or AHR. Differences in \dot{V}_i between periods *B* and *C* were used as measure of the hypoxic ventilatory decline or HVD. The \dot{V}_i responses are expressed as the change in \dot{V}_i per percentage change in $S_{p}O_2$ (units: $L \min^{-1} \%^{-1}$).

Statistical Analysis

The variance ratio test (*F* ratio test) was used to compare the goodness of fit among the one-

and two-compartment models. This test indicates whether, after allowing for the difference in the number of parameters between the nested models, the larger model still provides a statistically significant improvement in the fit to a common data sequence, compared with the smaller model. The F -statistic was calculated as follows:²

$$(5) \quad F = \frac{(RSS_1 - RSS_2)/(df_1 - df_2)}{RSS_2/df_2} \sim F(df_1 - df_2, df_2)$$

where RSS_1 and df_1 refer to the residual sum of squares and degrees of freedom of the smaller model and RSS_2 and df_2 refer to the residual sum of squares and degrees of freedom of the larger model. Note that the F -ratio assumes that the residuals are uncorrelated (white or close to white). This was obtained by modeling the noise using the parallel noise pathway.¹⁴⁴

On the parameters obtained from the two-compartment model we performed a paired (normoxia *versus* hypoxia) and unpaired (the effect of carotid body resection) analysis of variance.

The effect of CBR on the AHR and HVD was tested by one-way analysis of variance. Values are mean \pm SD. P -values < 0.05 were considered significant.

RESULTS

Hypercapnic Studies

An example of a ventilatory response to two subsequent MFBS CO_2 inputs of a bilateral CBR patient is given in figure 1. The fit of the two-compartment model to the data is given (line through the data points).

Model Comparison.

- For the normoxic CO_2 data in bilateral CBR patients the two-compartment model did not provide a statistically significant improvement over the one-compartment model. For the hypoxic CO_2 data an improvement occurred in 1 out of 7 subjects.
- For the unilaterally resected patients, the two-compartment model fitted the data significantly better than the one-compartment model for 6 out of 7 subjects under both conditions of normoxia and hypoxia.
- For control subjects, the two-compartment model fitted the data significantly better than the one-compartment model for 5 out of 7 subjects under both O_2 background conditions.

Model Parameters. The mean parameter values are given in table 1. The statistical analysis was performed on the parameters of the two-compartment in order to test the effect of the protocol for each of the three subjects groups (paired anova). For bilaterally CBR patients parameter G_P remained unaffected by hypoxia. The increase of G_P in hypoxia seen in unilaterally resected patients was not significant. Only in control subjects did G_P increase significantly with hypoxia ($P < 0.05$). All other parameters were unaffected by hypoxia, with the exception of G_C in control subjects which increased significantly ($P < 0.05$).

In order to investigate the effect of carotid body resection on model parameters, an unpaired anova was performed on the parameters of the two-compartment model as

Table 2. Model parameters of the two- and one-compartment models

	G_C	G_P	B_k	τ_C	τ_P	T_C	T_P	C
	$L \text{ min}^{-1} \text{ kPa}^{-1}$	$L \text{ min}^{-1} \text{ kPa}^{-1}$	kPa	s	s	s	s	ml min^{-2}
—Two-Compartment Model: Bilateral Carotid Body Resection—								
<i>normoxia</i>	7.1 ± 5.3	1.6 ± 1.2	4.5 ± 0.5	116 ± 71	11 ± 11	13 ± 8	9 ± 4	80 ± 40
<i>hypoxia</i>	7.1 ± 2.4	1.6 ± 1.8	4.6 ± 0.5	125 ± 92	13 ± 10	12 ± 6	9 ± 5	60 ± 70
—Two-Compartment Model: Unilateral Carotid Body Resection—								
<i>normoxia</i>	10.4 ± 3.3	2.2 ± 1.4	4.6 ± 0.4	103 ± 87	3 ± 6	9 ± 5	7 ± 2	120 ± 90
<i>hypoxia</i>	10.4 ± 5.1	3.6 ± 2.4	4.5 ± 0.6	114 ± 52	7 ± 7	10 ± 5	5 ± 2	100 ± 90
—Two-Compartment Model: Control—								
<i>normoxia</i>	10.3 ± 4.2	3.1 ± 2.2	4.9 ± 0.3	148 ± 69	6 ± 7	12 ± 5	6 ± 1	60 ± 60
<i>hypoxia</i>	12.2 ± 3.9	4.6 ± 2.4	5.0 ± 0.2	130 ± 61	7 ± 4	13 ± 6	5 ± 1	90 ± 40
—One-Compartment Model: Bilateral Carotid Body Resection—								
<i>normoxia</i>	7.9 ± 4.6	-	4.4 ± 0.5	87 ± 37	-	6 ± 2	-	90 ± 50
<i>hypoxia</i>	7.9 ± 2.5	-	4.5 ± 0.3	92 ± 87	-	7 ± 4	-	60 ± 70

Values are mean \pm SD; G_C and G_P are the ventilatory CO_2 sensitivity of the central and peripheral chemoreflex loops; B_k is the apneic threshold; τ_C and τ_P are the time constants of the central and peripheral chemoreflex loops; T_C and T_P are the time delays of the central and peripheral chemoreflex loops; C is a trend term.

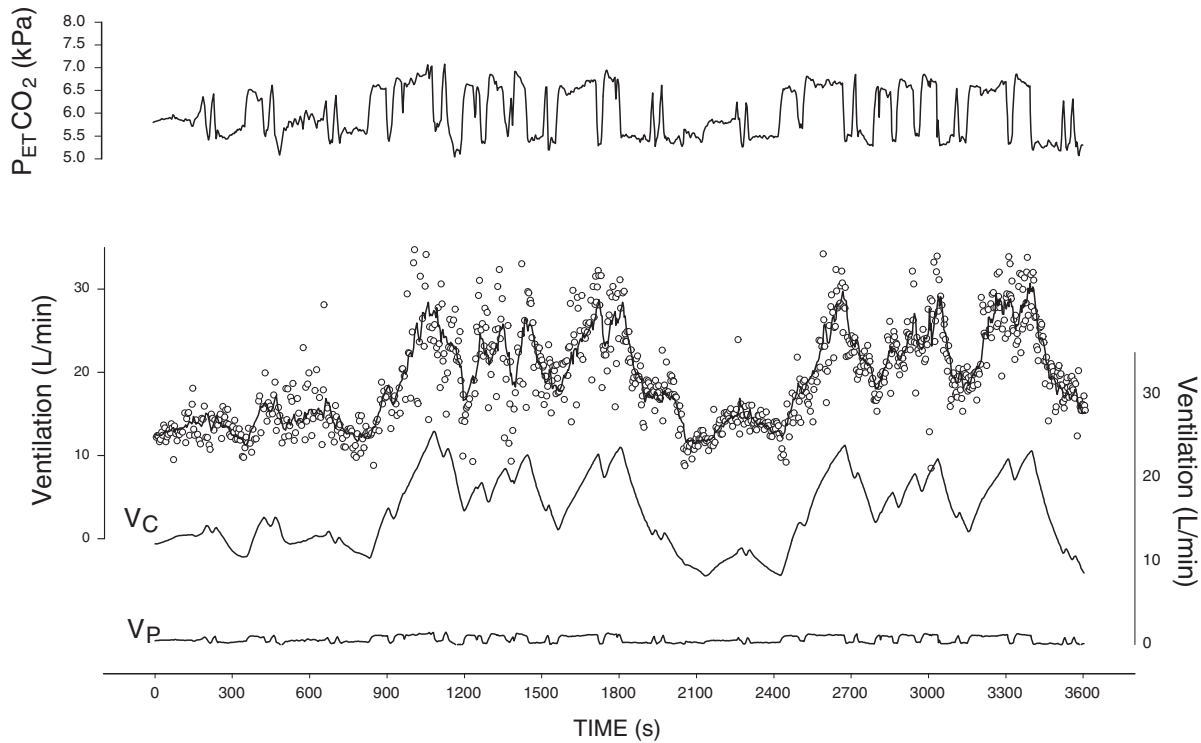


Figure 1. Two-compartment model fit to the normoxic CO_2 data of a bilaterally CBR patient. Shown is the response to 2 subsequent MFBS CO_2 inputs. Each dot is one breath. The line through the data points is the sum of the peripheral component (\dot{V}_P), central component (\dot{V}_C), parallel noise (\dot{V}_N), measurement noise (W) and a trend term (C). Only components \dot{V}_P and \dot{V}_C are shown.

a factor between the three subject groups. This effect showed a significant difference across groups of G_C and G_P ($P < 0.05$). This is, a lower G_C and G_P for the bilateral CBR patients compared to the unilateral CBR patients; and also a lower G_C and G_P for the unilateral CBR compared to control (see also fig. 2). There was also a significant decrease in B_k in both bilaterally and unilaterally resected patients compared with the control group ($P < 0.05$). There was no significant interaction between the carotid body condition and the protocol (hypoxia effect), probably due to the lack of hypoxic effect in unilaterally CBR patients, as observed in the paired comparison.

Inspection of the noise pathway revealed that successive breaths are less correlated in the absence of carotid bodies.

Hypoxic Studies

The acute hypoxic response increased significantly from bilaterally to unilaterally CBR patients and control subjects: 0.12 ± 0.09 , 0.53 ± 0.43 ($P = 0.03$ vs. bilateral CBR), and 1.33 ± 0.80 L min⁻¹ %⁻¹ ($P = 0.03$ vs. unilateral CBR and $P < 0.01$ vs. bilateral CBR). The magnitude of HVD did not differ among the three groups although there was trend towards a greater HVD with a greater AHR (fig. 3): bilateral CBR 0.35 ± 0.27 , unilateral CBR 0.46 ± 0.34 and control 0.83 ± 0.65 L min⁻¹ %⁻¹ ($P = 0.11$).

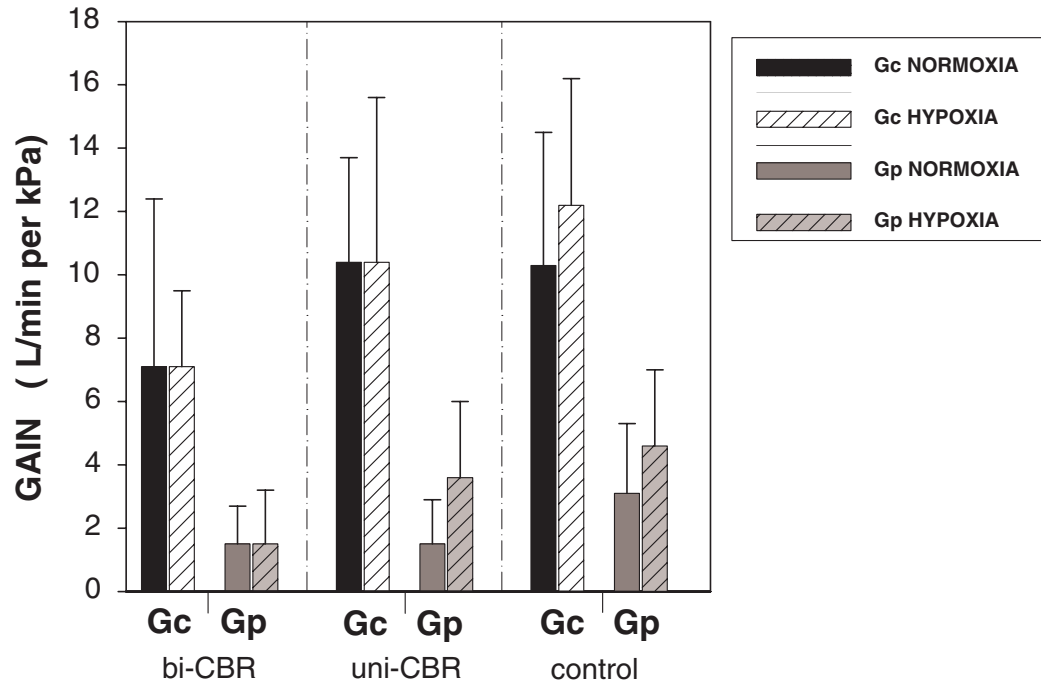


Figure 2. Mean values \pm SD of the gain's of the two-compartment model for bilateral and unilateral CBR patients and control subjects. See text for the result of the paired (effect of hypoxia) and unpaired comparisons (differences among the three groups).

DISCUSSION

This study provides additional data on human subjects who have undergone CBR. Our findings in otherwise healthy patients using MFBS CO_2 inputs to the ventilatory control system are in the general direction predicted from previous studies in humans and animals.^{12,40,55,78,91,208} The main finding of our study is the need for only a one-compartment model when fitting normoxic and hypoxic CO_2 data in patients after bilateral CBR (*i.e.*, the absence of a significant improvement in fit in the two-compartment model). When a significant improvement in fit does occur with the introduction of a second, fast component, it is associated with the presence of a peripheral chemoreflex response. This occurred in unilaterally CBR patients and control subjects. Our data indicate that the peripheral component (G_p) arises from the carotid body.

Central-Peripheral Ventilatory Chemoreflex Interaction

The value of G_c increased in hypoxia in control subjects (table 2). This may suggest central-peripheral interaction (that is, the modulation of the central gain of the respiratory controller by the peripheral drive from the carotid bodies). The finding that G_c increased from bilateral to unilateral CBR patients to control subjects (especially in hypoxia) is further proof for this form of interaction.

In the work of Bellville *et al.* there are some indications for such an interaction in the

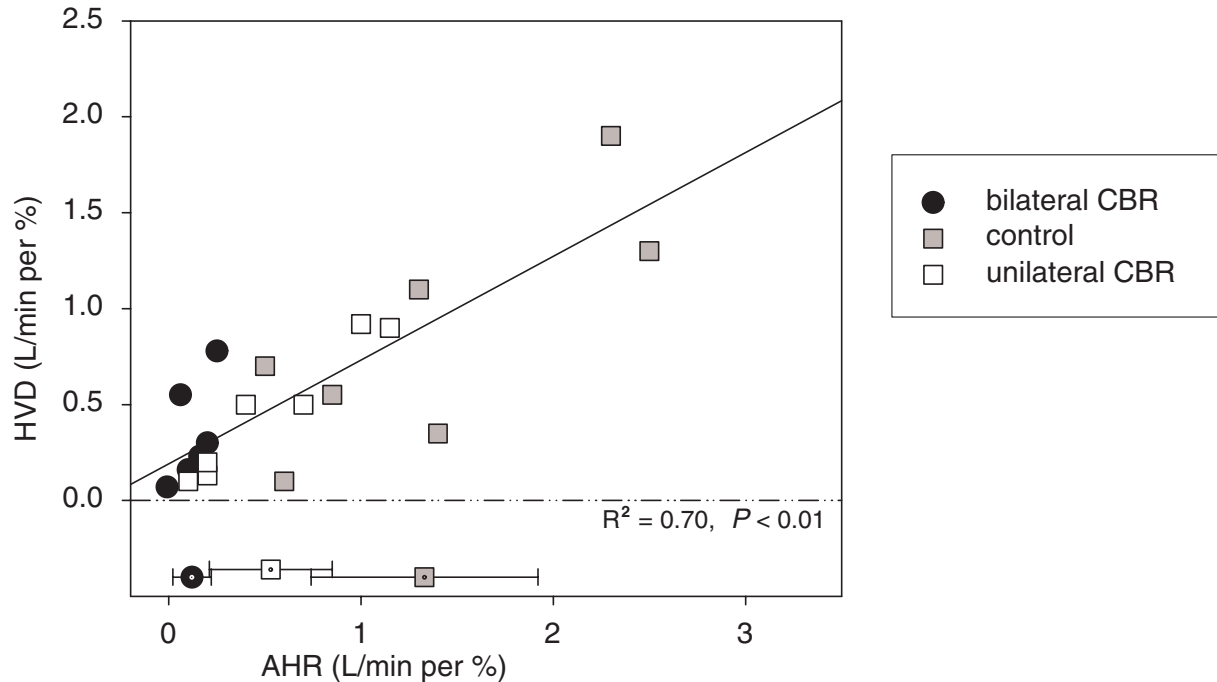


Figure 3. The acute hypoxic response (AHR) versus the hypoxic ventilatory decline (HVD). The continuous line is the linear regression. On the bottom the mean \pm 95% confidence interval AHR-values for the three groups is given.

human respiratory control system.¹² They found in normal subjects an increased central CO_2 sensitivity in hypoxia compared to normoxia and, like we did, in subjects who had undergone CBR a decreased CO_2 sensitivity was obtained. On the other hand, Ward & Bellville found no significant reduction of the central CO_2 sensitivity after intravenous infusion of dopamine, which caused a large decrease of the peripheral CO_2 sensitivity.²⁰⁹

Results of Robbins may also point into the direction of an interaction.¹⁵⁹ He compared hypoxic steps against a background of normocapnia at the peripheral chemoreceptors and initial hypercapnia at the central chemoreceptors with hypoxic steps against a background of normocapnia at both sets of chemoreceptors. Two of his three subjects showed an increased ventilatory response to steps into hypoxia when central PCO_2 was high. The issue of central-peripheral interaction has also been pursued by others using a similar protocol as that of Robbins.^{32,33,188} Their results do not lend much support for inclusion of central-peripheral interaction in the model of the chemoreflexes.

Dahan *et al.* observed the reduction of the central gain with hyperoxia which reduced G_p by $> 70\%$.⁴⁰ However, in an attempt to fit normoxic step CO_2 response curves using an central-peripheral interaction model, they observed that the model was overparameterized.

While our current study is entirely convincing regarding the origin of the peripheral, fast component, \dot{V}_p , the existence of central-peripheral interaction remains a challenging issue for further research. So far, the data confirming central-peripheral interac-

tion comes mostly from data involving CBR (*cf.* ref. 12 and this study). It may well be that after CBR transient or permanent changes in central chemoreflex function occurs (plasticity).¹³⁹ We followed one patient for over one year after CBR and observed a large but over time variable depression of G_C relative to pre-operative conditions. This suggests a change in the central chemoreflex loop which had not reached a steady-state as yet. Finally, our results as well those of others may direct also towards central O_2 - CO_2 interaction.

Characteristic of Components

We did observe an increase in G_P and AHR among the three groups, suggesting that each of the carotid bodies have an additive effect on the peripheral contribution to CO_2 - and hypoxia-stimulated breathing. Although the magnitude of the hypoxic ventilatory decline did not differ among the three groups, there was a clear trend of increased HVD with increased AHR (fig. 3). This suggests the need for AHR in the development of HVD. This is in agreement with a previous observation where we observed that despite central hypoxia (*i.e.*, within the CNS), but absence of peripheral drive, HVD did not develop.⁵⁰

The very small but significant AHR in bilaterally resected subjects was surprising (fig. 3) but may be due an effect of hypoxia on central O_2 -sensitive chemosensors.¹⁹⁸ Taken into account the CO_2 data, we do not believe that the small AHR reflects the return of peripheral chemoreception (*e.g.*, at the end of the cut sinus nerve or at arterial chemoreceptors).

We observed 0.3 to 0.5 kPa lower B_k values after uni- and bilateral CBR relative to control subjects. This suggest only a minor addition of the peripheral chemoreflex to ventilatory drive when the system is not stimulated by CO_2 . Whether this is also true under conditions other than the awake state (for example sleep or propofol anesthesia[†]) deserves further study.

The central chemoreflex gains in the unilaterally CBR patients and control subjects obtained under conditions of normoxia as well as all the other 'normoxic' parameters are in close agreement with previous observations in a group of healthy young volunteers (18–21 years).⁴⁰ This suggests the absence of age effect, at least over the age range studied, on the dynamics of the ventilatory control system.

In conclusion, we give additional proof that, in humans, the quantitative contribution of the peripheral and central respiratory chemoreflexes to CO_2 -stimulated breathing, under conditions of constant background $P_{ET}O_2$, (and the effect of pharmacological agents on these chemoreflexes) is reliably assessed using the two-compartment model of the ventilatory control system as previously suggested by Bellville *et al.*¹² and Dahan *et al.*⁴⁰

[†]see Chapters 4 and 5

3 Antioxidants prevent depression of the acute hypoxic ventilatory response by subanesthetic halothane

A MAJOR DEFENSE of the mammalian body to acute hypoxia is a rapid increase in pulmonary ventilation called the acute hypoxic response (AHR). This vital chemoreflex is primarily mediated by the carotid bodies located at the bifurcations of the common carotid arteries⁸⁰ During the past decade considerable progress has been made in unraveling the cascade of events within carotid body type I cells upon exposure to a hypoxic environment, although there are still many areas of controversy.^{80,118}

The general pictures emerging from most studies is that low oxygen decreases the open probability of potassium channels which causes membrane depolarisation and influx of Ca^{2+} ions. In several species, various types of potassium channels are described that may serve as oxygen sensing element that initiates the transduction cascade in hypoxia, for example K_V channels in rabbit,^{146,148} and Maxi-K and TASK channels in rat.^{25,158} Although it is known that potassium channels confer redox sensitivity and are sensitive to changes in the concentration of reactive oxygen species (ROS) it is unclear by what mechanism low oxygen is able to decrease the conductance of these channels.^{102,105,118}

Volatile anaesthetics such as halothane can open potassium channels in various cell types such as TASK channels in rat carotid body.^{25,143,184,140,141} At the same time, volatile anaesthetics, particularly halothane, are known to depress the acute hypoxic response, an effect that may be mediated through a preferential and potent action on the carotid bodies.^{100,47} It is unknown if opening of potassium channels by halothane might occur through changes in the cell redox state and/or changes in ROS. It is known, however, that during hypoxia halothane undergoes a reductive metabolism in the liver by which radical species are produced and lipid peroxidation is initiated; this reductive metabolism of halothane is thought to be responsible for its mild hepatotoxic effect.^{56,57,95,187} In guinea pig liver, peroxidation of lipids following halothane administration can be inhibited by antioxidant treatment with vitamin E.¹⁷⁷

The above findings on the sensitivity of potassium channels to ROS, the ability of halothane to produce radical species and to open potassium channels and finally the role of potassium channels in the hypoxic response raise the question if halothane may reduce the hypoxic response by producing ROS or by influencing the redox state of the carotid body. The aim therefore of the present studies in humans was to examine the influence of the potent antioxidants α -tocopherol and ascorbic acid on the acute hypoxic ventilatory response.

METHODS

Subjects and Apparatus

Thirty-two healthy, non-smoking, male subjects (age 20 to 35 yr) were recruited after protocol approval by the Leiden University Medical Center Committee on Medical Ethics. None of the volunteers was taking any medication or ever had surgery under general anaesthesia. All subjects performed a series of test experiments to familiarize them with the apparatus and experimental procedures. The subjects were instructed not to eat or drink for at least 8 hours prior to the study. They were not instructed about respiratory physiology, anesthesia and the intentions of the study. All gave oral and written informed consent before their participation.

After arrival at the laboratory, an intravenous catheter was inserted in the left or right antecubital vein for drug infusion. Subsequently electrodes for EEG monitoring (BisSensor, Aspect Medical Systems, Newton, MA) were placed on the head at AT₁-FP₁ as specified by the manufacturer, and the subjects rested for 20 to 30 min. Next a facemask was applied over the mouth and nose.

The EEG was recorded using an Aspect A-2000 EEG monitor (software version 3.3). The monitor computed the bispectral index (BIS), an objective measure of hypnosis,¹⁶⁴ over 2-s epochs. We averaged the BIS values over 1 min-intervals and used data points obtained at 3-min intervals for further analysis.

See METHODS section *Apparatus* of *Chapter 2* for a description of the procedure and apparatus. Part of the nitrogen (5 L/min) passed through a halothane vaporizer (Dräger 19·2, Lubeck, Germany). During the initial part of the study (control experiments), the vaporizer was kept in the "off"-position. Dräger Nederland BV calibrated the vaporizer prior to its use in this study.

Study Design

In the first set of studies, which was designed to test the effect of antioxidant pre-treatment on the depression by halothane of the acute hypoxic response (AHR), two separate groups of 8 subjects underwent a control hypoxic study, followed by a halothane hypoxic study, and finally by a halothane hypoxic study after pre-treatment with a cocktail of antioxidants (study 1) or placebo (study 2). In a second set of studies, which was designed to study the effect of antioxidant pre-treatment on the hypoxic ventilatory response in the absence of halothane, two separate groups of 8 subjects underwent a control hypoxic study, followed by a sham halothane hypoxic study, and next followed by a sham halothane study after pre-treatment with a cocktail of antioxidants (study 3) or placebo (study 4). While the design of the halothane administration was randomised and blinded to the subjects only, both subjects and researchers were blinded to the pre-treatment with anti-oxidants or placebo.

After each hypoxic study blood was drawn from the capillary bed of a hyperaemic finger for the determination of blood acidity (Åstrup equilibration technique, Radiometer, Copenhagen, Denmark).

The Hypoxic Study. Hypoxia was induced with a dynamic end-tidal forcing system:^{39,45} steps from normoxia ($P_{ET}O_2$ 15 kPa) into hypoxia ($P_{ET}O_2$ 6·2 kPa obtained within 4 to 6 breaths) were applied. Since peak hypoxic responses occur within three min,³⁹ hypoxia was maintained for three min, after which hyperoxia was introduced for 5 min ($F_iO_2 > 0·5$). The $P_{ET}CO_2$ was maintained just above individual resting values.

Halothane. During the appropriate studies, the subjects inhaled halothane (Fluothane, Zeneca

Ltd, Macclesfield, UK). By manipulating the settings of the vaporizer, the subjects inhaled 0.11% end-expiratory halothane for 10 min before the hypoxic study started. Inhaling 0.11% halothane for 10 min results in a MAC equivalent of 0.13 (assuming an age adjusted MAC of 0.84% in our young subjects).⁸³ Note that because of the short (10 min) exposure time to this end-tidal level of halothane, the brain concentration is less than 0.11%, preventing the occurrence of significant central effects (i.e., within the central nervous system) of halothane. The subjects were under the impression that halothane was given during the sham halothane studies by manipulating an empty vaporizer.

The Antioxidant Cocktail (AOX). The antioxidant cocktail consisted of 200 mg of oral α -tocopherol (Organon, Oss, The Netherlands) given 1-h prior to the start of the appropriate hypoxic study, which was ingested with a cup of yoghurt and two 1 gram intravenous doses of ascorbic acid (Ascorbinezuur CF, 5 ml, Centrafarm, The Netherlands) given 10 and 4 min before the appropriate hypoxic study. Placebos consisted of cellulose tablets and 0.9% NaCl manufactured by the local pharmacy). The oral placebo was also ingested with yoghurt.

Data and Statistical Analysis

Analysis was performed on a blinded data set. The breath-to-breath data of the last 10 breaths of normoxia and the last 10 breaths of hypoxia were averaged. Since the relationship between ventilation and arterial oxygen saturation is found to be linear,⁴⁵ we calculated the difference between the mean \dot{V}_i - and the S_pO_2 -data points and expressed the acute hypoxic ventilatory response (AHR) or sensitivity as follows:⁴⁵

$$\text{AHR} = [\dot{V}_i(\text{hypoxia}) - \dot{V}_i(\text{normoxia})] / [S_pO_2(\text{normoxia}) - S_pO_2(\text{hypoxia})]$$

(units L/min per % desaturation). The statistical analysis was performed using SPSS v10.0 for Windows. To detect the significance of differences among the three treatment groups of each study, a two-way analysis of variance was performed. *Post-hoc* analysis was by least-significant differences and Bonferroni tests. To assess the effect of antioxidant-versus placebo-pre-treatment, Student *t*-tests were performed on the appropriate treatment levels of studies 1 and 2 and studies 3 and 4. Values reported are mean \pm SD. *P*-values < 0.05 were considered significant.

RESULTS

All subjects completed the protocols without side effects. During all studies $P_{ET}CO_2$ values were kept constant 0.1 to 0.2 kPa above individual resting values, with no differences between baseline (pre-hypoxia) and hypoxic $P_{ET}CO_2$ values and pH. In all hypoxic studies S_pO_2 values were $82 \pm 2\%$.

The values of baseline ventilatory parameters and the control ventilatory responses to hypoxia are in agreement with earlier observations (table 1; refs. 48,45). We observed no effect from low dose halothane on baseline ventilation. Similarly, antioxidant and placebo pre-treatment had no significant effect on baseline parameters (table 1). Halothane (0.11% end-tidal) decreased the ventilatory response to hypoxia by more than 50%. As shown in figure 1, this effect was completely prevented by pre-treatment

Table 1. Influence of antioxidant and placebo pretreatment on halothane- and sham-halothane-induced depression of the ventilatory response to hypoxia

	STUDY 1			STUDY 2		
	control	halothane	AOX + halothane	control	halothane	placebo + halothane
Baseline \dot{V}_i (L/min)	12.1 ± 1.5	12.5 ± 3.3	14.0 ± 2.1	12.5 ± 1.6	12.7 ± 3.2	13.7 ± 4.1
$P_{ET}CO_2$ (kPa)	6.1 ± 0.4	6.1 ± 0.3	6.2 ± 0.2	6.0 ± 0.02	6.1 ± 0.2	6.0 ± 0.2
pH	7.41 ± 0.02	7.41 ± 0.02	7.42 ± 0.02	7.40 ± 0.02	7.40 ± 0.03	7.41 ± 0.02
Halothane ET vol.%	-	0.11 ± 0.01*	0.11 ± 0.01*	-	0.11 ± 0.01*	0.11 ± 0.01*
AHR (L min ⁻¹ % ⁻¹)	0.79 ± 0.31	0.36 ± 0.14*	0.77 ± 0.32	0.79 ± 0.40	0.36 ± 0.19*	0.36 ± 0.27*†
AHR (% of control)	100	46 ± 11*	96 ± 20	100	47 ± 14*	40 ± 15*†
BIS	96 ± 2	96 ± 2	96 ± 2	97 ± 1	97 ± 2	97 ± 1

	STUDY 3			STUDY 4		
	control	sham-halothane	AOX + sham halothane	control	sham-halothane	placebo + sham halothane
Baseline \dot{V}_i (L/min)	13.9 ± 1.9	14.5 ± 3.6	14.5 ± 2.8	14.6 ± 3.3	16.9 ± 3.8	16.1 ± 2.3
$P_{ET}CO_2$ (kPa)	5.8 ± 0.3	5.9 ± 0.2	5.8 ± 0.3	5.9 ± 0.4	5.9 ± 0.4	5.9 ± 0.4
pH	7.43 ± 0.03	7.43 ± 0.02	7.43 ± 0.03	7.42 ± 0.03	7.42 ± 0.02	7.41 ± 0.02
Halothane ET vol.%	-	-	-	-	-	-
AHR (L min ⁻¹ % ⁻¹)	0.89 ± 0.42	0.90 ± 0.44	1.00 ± 0.54	0.83 ± 0.42	0.88 ± 0.45	0.88 ± 0.45
AHR (% of control)	100	102 ± 14	116 ± 22	100	104 ± 15	110 ± 10
BIS	97 ± 2	97 ± 2	97 ± 1	96 ± 2	96 ± 3	96 ± 3

Values are mean ± SD; pH values are obtained during air breathing; BIS is bispectral index of the EEG;

AHR is Acute Hypoxic Response;

* = P < 0.01 vs. control of identical study (two-way analysis of variance);

† = P < 0.01 vs. AOX-pretreated halothane run on study 1 (Student-t-test).

with the antioxidant cocktail (study 1) but not by placebo pre-treatment (study 2). Sham halothane did not affect any of the ventilatory baseline and hypoxic parameters, neither did antioxidant (study 3) or placebo (study 4) pre-treatment (table 1 and fig. 2)). The 95% confidence intervals of antioxidant effect relative to halothane or sham-halothane (ratio AOX+halothane/halothane in study 1, and ratio AOX+sham halothane/sham halothane in study 3) did not overlap: 1.7, 3.1 and 0.6, 1.1 in studies 1 and 3, respectively (figure 3). This indicates that the effect of AOX to abolish halothane's depressant effect cannot be explained by an increase of the AHR by the antioxidants *per se*.

Bispectral index values did not differ among control, halothane, sham-halothane, antioxidant pre-treatment and placebo pre-treatment studies (table 1), indicating that there were no differences in the subjects' level of arousal across the various runs of all four studies.

DISCUSSION

We have found that while an antioxidant cocktail had only a small, statistically not significant, effect on the acute hypoxic response (fig. 3), it did reverse the large depression in the hypoxic response caused by low dose halothane. To place this result into context, we need to discuss methodological considerations; the modulating role of reactive oxygen species (ROS) in the chemoreception process; and the mechanism by which halothane depresses the hypoxic ventilatory response and how this effect might depend on the redox state in (the membrane of) chemoreceptors cells. The measurement of the hypoxic ventilatory response requires isocapnia both across drug treatments as well as during the hypoxic test. As seen in table 1 the mean differences in $P_{ET}CO_2$ for the different treatment conditions in the four studies were closely matched and did not contribute to the changes in the measured AHR.

Although we attempted to achieve blinding, the subjects were probably aware of when the halothane was being inhaled. The depression, however, of the AHR by halothane is large and consistent across subjects (fig. 1) while the changes in the AHR with the sham-halothane are variable and similar to the variation expected with repeated hypoxic tests. In testing the effects of inhalational anaesthetics, the experimental conditions are very important. We have previously shown that arousing the subject with audio visual stimulation can reverse the depression of the AHR by isoflurane.²⁰⁰

In the present experiments, the subjects were awake but left undisturbed. Because we did not see any influence of either halothane or the antioxidants on the bispectral index (table 1), we have no observational evidence of an influence of the subject's level of arousal on our results. While we believe that the antioxidant cocktail that we utilized was effective in altering the intracellular or extracellular redox state, we have no direct measurement of their efficacy in our subjects. We rationalize the use of an antioxidant cocktail as follows. We had to take into account that the effects of halothane could be located at several sites: at the outer face of the membrane, within the membrane or in the cytosol or possibly at the mitochondrial level. For this reason we used the water-

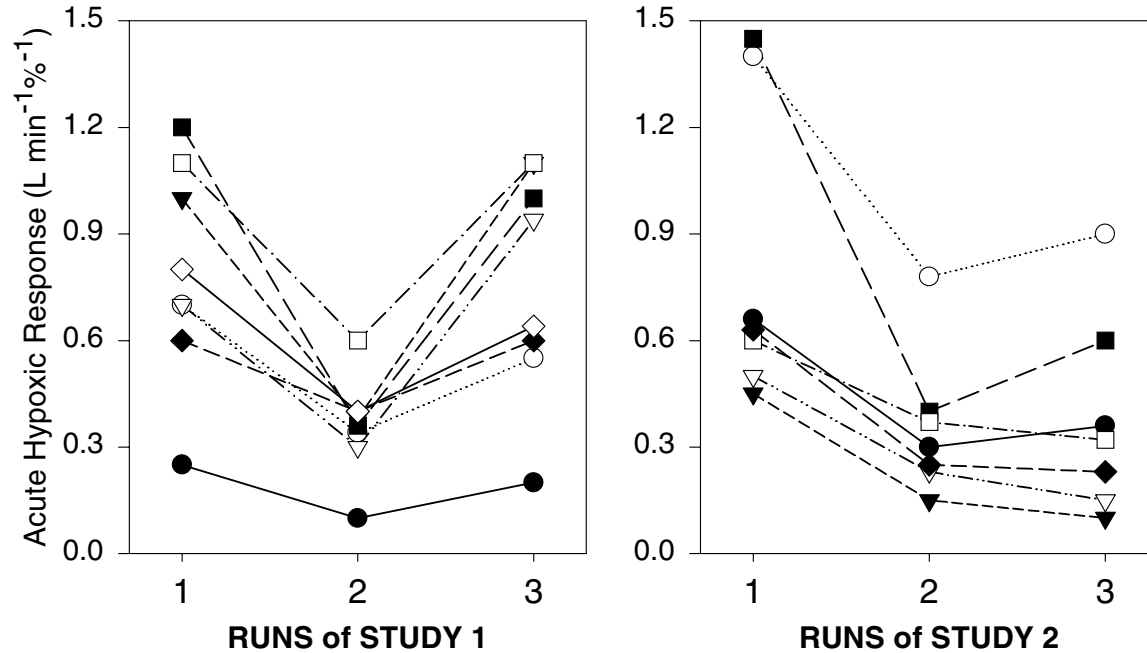


Figure 1. Hypoxic ventilatory responses of individual subjects of studies 1 and 2. Study 1: Control, run 1, and halothane hypoxic ventilatory responses, run 2, and influence of antioxidant, run 3 pretreatment on halothane-induced impairment of the hypoxic drive. Study 2: Control, run 1, and halothane hypoxic responses, run 2, and influence of placebo pre-treatment on halothane-induced impairment of the hypoxic drive, run 3. Note the ability of antioxidant but not placebo pre-treatment to prevent depression of the hypoxic response by halothane.

soluble ascorbic acid which is a particularly potent anti-oxidant in plasma and in the cytosol^{27,74} and α -tocopherol which, due to its lipid solubility, may be the most important free radical and lipid peroxide scavenger in membranes.²⁶ Furthermore, it is known that the combined effectiveness of ascorbate and α -tocopherol is synergistic, with the net result that radicals originating from the membrane are removed using two different antioxidants.^{134,138} Combined administration of α -tocopherol (2000 I.U. i.m.) and ascorbic acid (2 g i.v.) has been shown to reduce lipid peroxidation in patients undergoing cardiac bypass operation.⁹ The oxygen transduction cascade in the carotid body (as in the similarly oxygen sensitive pulmonary artery smooth muscle and the pulmonary neural epithelial cell bodies) has been subject to considerable research over the past decade and while a much clearer picture of the process has emerged, there are many areas of considerable controversy.^{80,118} The most generally accepted model is that low oxygen decreases the open probability of potassium channels in the membrane of carotid body type I cells which results in depolarisation. This membrane depolarisation opens voltage gated calcium channels with the resulting influx of Ca^{2+} causing neurotransmitter release, which activates the synaptically adjacent carotid sinus nerve. Currently, much interest has focused on the oxygen sensitive potassium channels in the carotid bodies

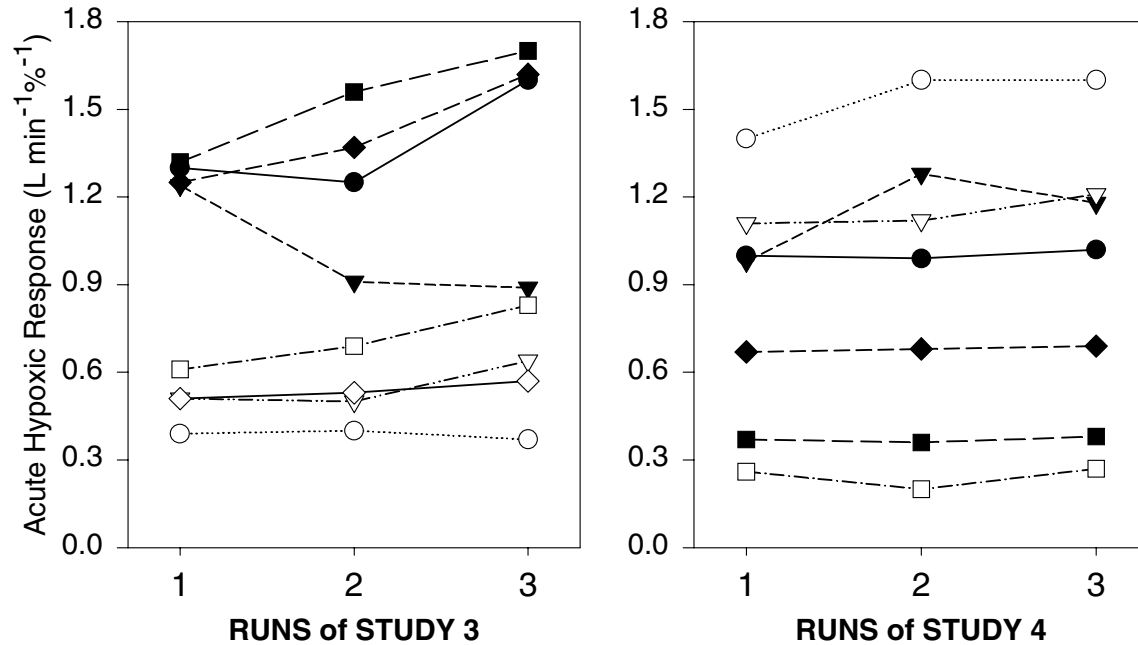


Figure 2. Hypoxic ventilatory responses of individual subjects of studies 3 and 4. Study 3: Control, run 1, and sham halothane hypoxic ventilatory responses, run 2, and influence of antioxidant pretreatment on the hypoxic drive during inhalation of sham halothane, run 3. Study 4: Control, run 1, and sham halothane, run 2, and the influence of placebo pre-treatment on the hypoxic drive during inhalation of sham halothane, run 3.

of several species.^{149,113} The rat and the rabbit have been most commonly studied and they appear to have different types of oxygen sensitive potassium channels. The rat appears to have both TASK,²⁵ and Maxi-K channels,¹⁵⁸ that are oxygen sensitive, while in the rabbit K_V channels seem to serve this role.^{146,148} However, within this general model, it is not determined how low oxygen closes the potassium channel that seems to initiate the cascade. Several studies have shown that potassium channels show redox sensitivity and considerable sensitivity to levels of ROS.^{102,105,142,117} It is unsettled whether potassium channels possess intrinsic oxygen sensitivity, or, alternatively, are influenced or modulated by other O_2 sensing elements in the cascade, for example by (membrane associated) cytosolic redox couples. Intrinsic oxygen sensitivity could exist in the form of reduction/oxidation of thiol containing free cysteine residues in b sub-units that are required for hypoxic sensitivity.¹⁴⁷ One proposed redox model associated with enzymatic production of ROS that may influence potassium channel conductance is the cytochrome P-450 system that utilizes NAD(P)H as an electron donor. Inhibition of this enzyme system has been shown to prevent the hypoxic inhibition of potassium channels⁸⁷ but this has not been found in all model systems.¹⁶⁵

It is clear that within this general framework of hypoxic chemoreception there is considerable variety in specific sensor elements and couplings. Particularly when channels

are expressed in heterologous systems, all the elements for the *in vivo* cascade may not be present. In addition, there may be substantive differences between sensing elements of the cascade between the different oxygen sensitive tissues. Thus, it has been difficult to verify the role for ROS in carotid body chemotransduction in more physiologically intact preparations. In fact, there is considerable controversy as to whether ROS increases (pulmonary arterial smooth muscle)^{111,214} or decreases (carotid body)¹⁰⁸ with hypoxia in oxygen sensitive cells. Experiments in which the redox state of carotid body cells was altered would seem to indicate that ROS may not be a direct link between hypoxia and the membrane depolarisation initiated by the closure of the K⁺ channel.^{165,167} Exogenous reductants, on the other hand, have been shown to mimic the effect of hypoxia on O₂ sensitive potassium channels in carotid body cells.¹¹ Thus, whatever the precise mechanism, there is likely to be at least a modulating role for the redox state of the type-I cell in O₂ sensing. The depressant effect of subanesthetic halothane in humans on ventilation during hypoxia may occur via a preferential and potent action on the carotid bodies.^{47,100} The mechanism for this depression is unknown but inhalational anaesthetics can directly open two-pore domain potassium (TASK) channels in various cell types,^{140,142,143,184} and in particular in the rat carotid body.²⁵ The action of inhalational anaesthetics on TASK channels may be located at a specific region at the junction between the final transmembrane domain and the cytoplasmic C-terminus.^{143,192} This site is also involved in neurotransmitter inhibition of the channel but does not contain a motif that is known to be involved in cell signalling mechanisms.¹⁹² How changes in ROS or redox state could alter the properties of this binding site is unknown. In the lung carcinoma cell line H146, a representative model for pulmonary oxygen-sensitive neuroepithelial body cells, halothane transiently reverses hypoxic inhibition of potassium currents, similar to the reversal caused by the reactive species H₂O₂.⁸⁶ The metabolism of halothane itself may also change the redox status of cells. In hypoxia, halothane undergoes a reductive metabolism that in the liver is catalysed by isoforms of cytochrome P450 but in other tissues possibly also by other heme-containing proteins.^{57,95,187} Reduction of halothane yields CF₃CHCl radicals able to inactivate cytochrome P450 by covalent binding, or, alternatively, to remove hydrogen from polyunsaturated lipids thus initiating lipid peroxidation.^{56,95} In guinea pig, the hepatotoxic effect caused by this reductive metabolism of halothane can be prevented by antioxidant treatment.¹⁷⁶ In humans, induction with hemin of heme oxygenase-1, which has an antioxidant role in oxidative stress, has been shown to be effective against halothane-induced liver damage.¹³⁶ The susceptibility of halothane's depressant effect to antioxidant treatment that we found in this study indicates that the cellular redox state influences the effect of halothane on the oxygen sensing mechanism. This could be explained by a modulation by ROS of the coupling of halothane to the potassium channel (or other channels). Whether or not the ROS was generated from halothane's metabolism or from other intracellular processes,²¹⁴ the reduction in ROS with antioxidant treatment could reduce the coupling of halothane to the channel and prevent it from opening it. An alternative way to explain our findings would be to suggest that an increase in the concentration of ROS has an inhibitory effect on the mechanism involved in the acute hypoxic response. In

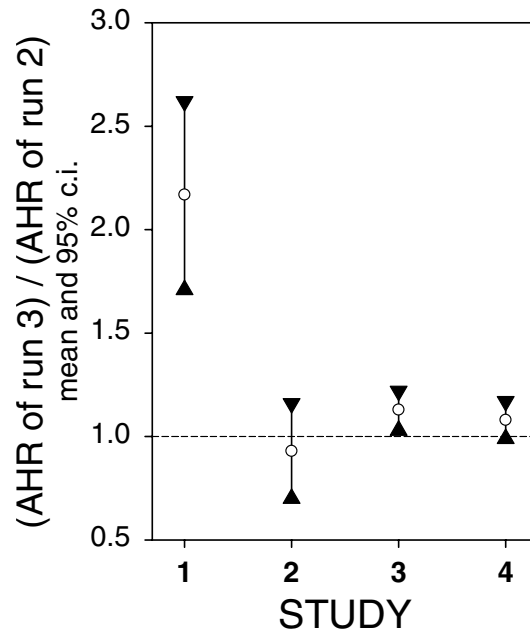


Figure 3. The effect of the antioxidant cocktail or placebo on halothane or sham-halothane induced depression of the acute hypoxic response. Values are the ratio of the third hypoxic run (antioxidants or placebo) over the second hypoxic run (halothane or sham halothane) of studies 1 to 4. \circ is mean, the triangles depict the 95% confidence intervals (c.i.). A value of 1 indicates no effect of the AOX or placebo pre-treatment on the acute hypoxic ventilatory response. Note that the 95% c.i.'s of studies 1 (AOX + halothane) and 3 (AOX + sham-halothane) do not overlap.

this scenario, the cellular redox state or the signalling from a particular ROS would be the coupling from low oxygen to potassium channel closure. In this model, an NAD(P)H oxidase has been proposed as the membrane bound source of oxygen sensitive ROS implying a decrease in ROS in hypoxia.^{94,96,106,181} The increase in local ROS caused by the reductive metabolism of halothane in hypoxia would thus counter the hypoxia-induced decrease in ROS and prevent the hypoxic closure of the K^+ channel. This effect would be most noticeable in hypoxia since halothane's reductive metabolism is increased in hypoxia.

In animal species, the effect of halothane on the hypoxic ventilatory response is variable. In the goat, for example, an end-tidal concentration of 0.5% does not significantly depress it.¹⁰⁴ In the rabbit and cat, 0.5-1% halothane reduces the hypoxic response, the effect in the latter species being larger.^{52,150} As shown in this and previous studies, the effect of 0.1 MAC in man is to reduce hypoxic sensitivity by more than 50%. These species differences could originate from the differences in the type of oxygen sensitive potassium channel that initiates the transduction cascade (e.g., TASK versus K_V) and their differences in anaesthetic sensitivity or in splice variants of the expressed channel. An alternative explanation could also lie in species differences in the defence against ROS. Goats produce large quantities of ascorbic acid,³¹ and may thus be better protected against the adverse effects of free radicals produced by halothane. To a

lesser degree this may also be the case for rabbits. Cats produce low quantities of ascorbic acid,³¹ and this might explain their higher susceptibility to halothane than rabbits. Humans have lost the ability to synthesize ascorbic acid and may therefore be more vulnerable to the adverse effects of reactive species that are produced by halothane. It is worth mentioning that in a previous study we were not able to demonstrate a clear depression of the normocapnic AHR by desflurane.⁴⁵ This volatile anaesthetic has a low metabolism, with little production of free radicals.¹⁰³ In *Chapters 4 and 5* we show that low dose propofol, which is known to have antioxidant properties,⁵⁸ neither depressed the CO_2 sensitivity of the peripheral chemoreflex loop nor the fast - carotid body mediated - component of the acute hypoxic response. Together with the present findings, these previous data suggest that the (lack of) depressant effects of anaesthetics on the hypoxic response may be related to their pro-oxidant (antioxidant) properties, but further studies are needed to support this hypothesis. From the data that we presented in this study we conclude that changing the cellular redox state can modulate the depressant effect of halothane on the acute hypoxic response. Further, although our results do not supply direct evidence for an inhibitory role of ROS in the acute hypoxic response, they could be explained by ascribing at least a modulating role to radical species in the AHR. Our observation that anti-oxidant pre-treatment markedly reduces the depressant effect of halothane on the AHR demonstrates a specific pharmacological reversal of an anaesthetic effect. Further work will be needed in both humans and animal preparations to clarify the interaction of cellular redox status, inhalational anaesthetics and oxygen sensitive potassium channels in the carotid body.

SECTION 2

Pharmacology

4 Propofol for monitored anesthesia care: *implications on hypoxic control of cardiorespiratory responses*

DURING MONITORED anesthesia care, in which anesthetics or analgesics are used as adjuvant to regional anesthesia, ventilatory control is predominantly metabolic or chemical in nature. Because hypoxia (especially episodic or intermittent hypoxia) is frequently associated with monitored anesthesia care,^{168,186} we investigated the influence of propofol, a popular hypnotic for sedation during such care, on the ventilatory responses to acute, sustained, and episodic isocapnic hypoxia.

Hypoxia has a dual effect on the ventilatory control system. A short episode of hypoxia (duration < 5 min) causes an increase in the hypoxic drive from the peripheral chemoreceptors of the carotid bodies.²⁰⁶ Hypoxia of longer duration causes depression of the respiratory centers in the brain stem.^{206,210} The net effect of these opposing phenomena is that the ventilatory response to sustained hypoxia is biphasic: an initial period of hyperventilation, the acute hypoxic response (AHR), is followed within 3 to 5 min by a slow hypoxic ventilatory decline (HVD). A steady-state in ventilation (\dot{V}_i) is obtained after 15 to 20 min.^{50,67} The mechanism of the hypoxic depression of \dot{V}_i (HVD) is unknown. During moderate hypoxia (oxygen saturation 80–90%) the accumulation and release of inhibitory neurotransmitters or modulators, such as γ -aminobutyric acid (GABA) or adenosine, is thought to play a major role in the development of HVD.^{49,50,67,68,124} A consequence of the hypoxia-related central depression is that the recovery of the hypoxic response is not immediate (i.e., after prolonged hypoxia subsequent hypoxic responses remain depressed).^{50,67}

Recent studies indicate that several general anesthetics, including propofol, interact with or modulate the GABA_A receptor complex.^{51,107,126} At clinical concentrations, propofol enhances GABA-evoked chloride currents and causes direct activation of the receptor in the absence of GABA.^{51,107} GABA_A receptors are thought to be involved in the generation of HVD,^{49,50,67,206,210} and hence there may be an important role for propofol in modulating/enhancing HVD.

Clinically, hypoxia is often episodic or periodic, especially during sleep or sedation.^{162,186} We therefore further examined the interaction of propofol and periodic hypoxia on \dot{V}_i and the development of HVD. Apart from assessing ventilatory responses we measured heart rate (HR) responses and the bispectral index (BIS) of the electroencephalogram (EEG). The BIS will inform us on the central nervous system arousal state of the participants, which is relevant because it may be an important factor in the study outcome.

METHODS

Subjects and Apparatus

Ten healthy male volunteers (aged 18–25 yr) participated in the protocol after approval was

obtained from the Leiden University Medical Center Human Ethics Committee. The subjects were healthy and did not have a history of tobacco or illicit drug use. They were instructed not to eat or drink for at least 8 h before the study.

After arrival at the laboratory, two intravenous catheters were inserted in the left and right cubital vein (one for propofol administration and one for blood sampling). Subsequently standard electrodes for EEG measurement (BisSensor, Aspect Medical Systems, Natick, MA) were placed and the subjects rested for 20 to 30 min. Next a face mask was placed and the experiments started.

See METHODS section *Apparatus* of *Chapter 2* for a description of the procedure and apparatus. The EEG and electromyogram were recorded using an Aspect (Natick, MA) A-2000 EEG monitor. The monitor computed the bispectral index (BIS) over 4-s epochs. We averaged the BIS values over 1 min-intervals.

Study Design

Two different hypoxic tests (sustained hypoxia and hypoxic pulses) were performed without and with propofol. Control studies preceded propofol studies. The order of hypoxic tests was randomized. Between tests there was ample time for resting. Control and drug hypoxic studies were performed at identical $P_{ET}CO_2$'s, 5–7 mmHg above awake resting values. This was done to balance an effect of the increase in $P_{ET}CO_2$ resulting from depression of \dot{V}_i by propofol.

Sustained Hypoxic Test. The $P_{ET}O_2$ was forced as follows: (1) 10 min at 110 mmHg, (2) a rapid decrease to 50 mmHg, (3) 15 min at 50 mmHg, (4) a rapid increase to 110 mmHg, (5) 2 min at 110 mmHg, (6) a rapid decrease to 50 mmHg, (7) 3 min at 50 mmHg, (8) at least 5 min at more than 300 mmHg.

Hypoxic Pulses. The $P_{ET}O_2$ waveform was as follows: (1) 10 min at 110 mmHg, (2) a rapid decrease to 50 mmHg, (3) 3 min at 50 mmHg, (4) a rapid increase to 110 mmHg, (5) 2 min at 110 mmHg. The hypoxic-normoxic sequence (steps 2–5) was repeated five times. This procedure yields six 3-min hypoxic pulses separated by 2 min of normoxia.

Propofol Administration, Sampling and Assay. A Psion (London, United Kingdom) palm-top computer programmed with a three compartment propofol pharmacokinetic data set,³⁴ was used to control a Becton Dickinson infusion pump (St. Etienne, France) for the intravenous administration of propofol. The propofol target concentration was set at 1 μ g/ml and was kept constant during both propofol hypoxic studies. After BIS values had reached a steady level, but at least twenty minutes after the target had been reached, the hypoxic studies started. Propofol blood samples (5 ml) were obtained 5 min before the first hypoxic study (T_1), at the end of the first hypoxic study (T_2) and at the end of the second hypoxic study (T_3). The samples were collected in syringes containing potassium oxalate. The propofol concentrations were determined by reverse-phase high performance liquid chromatography.²⁰⁷

Data Analysis

Sustained Hypoxic Test. Mean values of the breath-to-breath data were chosen over identical time segments (see figure 1). Period N is the 1-min period before the 15-min of hypoxia; period H_1 the 3rd min of hypoxia; period H_2 the 15th min of hypoxia; and period H_3 the 3rd min of the second hypoxic bout. Differences in \dot{V}_i between Periods N and H_1 were defined as the first AHR (AHR_1), between Periods N and H_2 as the sustained hypoxic response, and between Periods N and H_3 as the second AHR (AHR_2). The difference between AHR_1 and sustained hypoxic

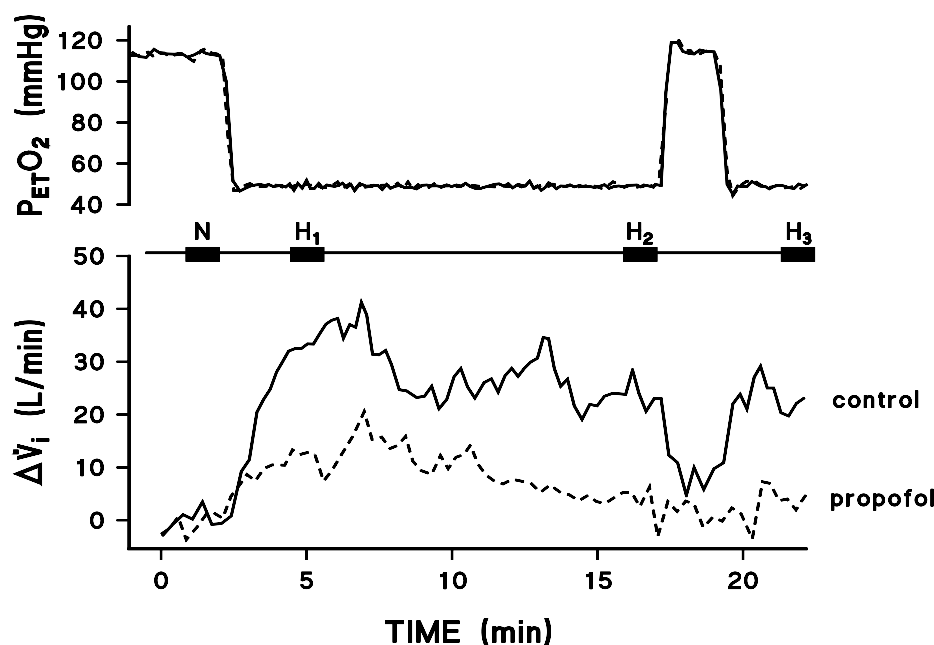


Figure 1. Control and propofol ventilatory responses to the sustained hypoxic test of one subject. *TOP*. End-tidal pressure of oxygen input to the subject. *BOTTOM*. Ventilatory responses above normoxic baseline (\dot{V}_i data are averaged over six breaths). Period N is the 1-min period before the 15 min of hypoxia; period H₁ min 3 of hypoxia; period H₂ min 15 of hypoxia; and period H₃ min 3 of the second hypoxic bout. Continuous line = control; dashed line = propofol.

response was used as measure of the HVD. The \dot{V}_i responses are expressed as the change in \dot{V}_i per percentage change in S_pO_2 (unit: $L \min^{-1} \%^{-1}$).

Hypoxic Pulses. We tested the occurrence of HVD by comparing the hypoxic response to the first hypoxic pulse with the response to the last hypoxic pulse. In order to do so, mean values of the breath-to-breath data of the last min of normoxia before the first hypoxic pulse (period A) and the third min of the first (period B) and last hypoxic pulse (period C) were calculated. Differences in \dot{V}_i between periods A and B were defined as the first AHR (AHR_{first}), and between periods A and C as the last AHR (AHR_{last}).

Statistical Analysis

A two-way analysis of variance was performed on the different periods (N, H₁, H₂ and H₃) of the sustained hypoxic test. Because peak heart rate responses occurred at period H₁ + 3 min (see Results), for hypoxic HR sensitivities a comparison was made among periods H₁ + 3 min, H₂ and H₃. Differences between periods were tested with the Student-Newman-Keuls test. A paired-*t*-test was performed to compare ventilatory and heart rate responses to the first and sixth hypoxic pulse. To detect the significance of difference between the control and propofol studies, a paired-*t*-test was performed on individual parameters of the hypoxic studies. *P* values < 0.05 were considered significant. All values are mean \pm SD.

RESULTS

Propofol Concentrations and BIS Values.

Blood propofol concentrations and BIS values varied considerably among subjects but were constant over time within subjects. Over time, blood propofol concentrations were $0.61 \pm 0.30 \mu\text{g/mL}$ at T_1 , $0.56 \pm 0.23 \mu\text{g/mL}$ at T_2 , and $0.58 \pm 0.23 \mu\text{g/mL}$ at T_3 (not significant). Mean coefficient of variation over time was 14.6% (range 7–34%). Because of technical problems, the collection of BIS data was not achieved in one subject. Averaged control BIS values were 97 ± 2 and 96 ± 2 for the sustained hypoxic and hypoxic pulses studies, respectively. During propofol, the mean BIS values were 76 ± 14 for the sustained hypoxic study (range among subjects 53–91; mean coefficient of variation over time = 7%), and 76 ± 10 for the hypoxic pulses study (range among subjects 66–91; mean coefficient of variation over time 8%).

Ventilatory Responses

Sustained Hypoxic Test. Propofol reduced normoxic baseline \dot{V}_i by about 15% ($P < 0.001$; table 1). In figure 1, examples of a control and propofol hypoxic study of one subject are shown. In all subjects, in both control and propofol studies, the hypoxic ventilatory responses were biphasic and the recovery of the hypoxic response was not immediate (figures 1, 2, table 1). Propofol decreased AHR_1 by 50% from 1.74 ± 1.22 to $0.89 \pm 0.70 \text{ L min}^{-1} \%^{-1}$ ($P < 0.001$), the sustained hypoxic response by 60% from 1.11 ± 0.80 to $0.33 \pm 0.30 \text{ L min}^{-1} \%^{-1}$ ($P = 0.002$) and AHR_2 by 60% from 1.04 ± 0.48 to $0.39 \pm 0.33 \text{ L min}^{-1} \%^{-1}$ ($P < 0.001$). The absolute magnitude of HVD did not differ between control and propofol (0.63 ± 0.51 versus $0.56 \pm 0.52 \text{ L min}^{-1} \%^{-1}$, not significant). Propofol increased the ratio HVD/AHR_1 by more than 50% from 0.35 ± 0.16 to 0.54 ± 0.16 ($P = 0.02$), and caused more depression of the second hypoxic response: The ratio $\text{AHR}_2/\text{AHR}_1$ was 0.67 ± 0.16 for control and 0.51 ± 0.22 for propofol ($P < 0.05$).

Hypoxic Pulses. BIS values and control of end-tidal gas concentrations are listed in table 2. In figure 3, examples of a control and propofol study of one subject are shown. In the control study, \dot{V}_i in Periods B and C increased to $197 \pm 78\%$ and $200 \pm 63\%$ of baseline, respectively. Corresponding values in the propofol study were $154 \pm 27\%$ (period B) and $144 \pm 37\%$ (period C). In control and propofol studies $\text{AHR}_{\text{first}}$ did not differ from AHR_{last} : control 1.35 ± 0.84 versus $1.35 \pm 0.67 \text{ L min}^{-1} \%^{-1}$ (not significant; figure 4); propofol 0.64 ± 0.39 versus $0.58 \pm 0.25 \text{ L min}^{-1} \%^{-1}$ (not significant).

Heart Rate Responses

Because of technical problems, the collection of heart rate data failed in one subject. Propofol decreased normoxic HR by 8–10 beats/min (table 1). In control and propofol sustained hypoxic studies, peak heart rate responses occurred at period $H_1 + 3 \text{ min}$ (figure 2). Control heart rate sensitivity decreased from its peak by 20% in period H_2 (not significant) and by 35% in period H_3 ($P < 0.05$). Propofol heart rate sensitivity

Table 1. The Ventilatory, Heart Rate and Bispectral Index Responses to Sustained Hypoxia before and during Propofol Administration

		Period N	Period H ₁	Period H ₂	Period H ₃
\dot{V}_i (L/min)	control	19.2 ± 5.2	37.6 ± 14.4*	31.0 ± 11.3†	30.8 ± 11.1†
	propofol	16.0 ± 8.4	25.4 ± 13.3*	19.8 ± 10.0†	20.4 ± 10.9†
\dot{V}_i (% of baseline \dot{V}_i)	control	100	195 ± 54*	162 ± 28†	158 ± 21†
	propofol	100	160 ± 29*	127 ± 16†	127 ± 10†
V_T (mL/breath)	control	1145 ± 201	1755 ± 296*	1578 ± 305†	1559 ± 324†
	propofol	913 ± 368	1287 ± 426*	1104 ± 373†	1097 ± 371†
RR (breaths/min)	control	17 ± 4	22 ± 10*	20 ± 6	20 ± 6
	propofol	16 ± 3	19 ± 5*	17 ± 3‡	18 ± 4‡
Heart Rate (beats/min)	control	69 ± 10	83 ± 16*	83 ± 14*	81 ± 12*
	propofol	58 ± 11	68 ± 16*	68 ± 15*	66 ± 14*
BIS	control	96 ± 2	97 ± 1	97 ± 2	97 ± 2
	propofol	78 ± 11	79 ± 9	74 ± 19	74 ± 17
P_{ETCO_2} (mmHg)	control	46.8 ± 2.9	46.6 ± 3.0	46.9 ± 3.0	46.7 ± 2.9
	propofol	46.6 ± 2.7	46.5 ± 3.1	46.8 ± 2.9	46.7 ± 2.9
P_{ETO_2} (mmHg)	control	112.0 ± 0.8	49.1 ± 0.8*	49.1 ± 0.4*	49.0 ± 0.8*
	propofol	112.8 ± 1.1	49.1 ± 0.5*	48.4 ± 0.4*	49.0 ± 0.7*
S_pO_2 (%)	control	98 ± 1	87 ± 4*	87 ± 4*	87 ± 4*
	propofol	98 ± 1	86 ± 4*	85 ± 3*	86 ± 3*

Values are mean ± SD.

N = the last normoxic min before the 15-min hypoxic period; H₁ = min 3 of initial hypoxia;

H₂ = min 15 of initial hypoxia; H₃ = min 3 of the 2nd hypoxic episode.

* $P < 0.05$ versus Period N. † $P < 0.05$ versus Period N and H₁. ‡ $P < 0.05$ versus Period H₁.

decreased from its peak by 21% in period H₂ ($P < 0.05$) and by 38% in period H₃ ($P < 0.05$). Compared with control, propofol decreased hypoxic heart rate sensitivities in periods H₁ + 3 min, H₂ and H₃ by 27 ± 36%, 36 ± 22 % and 38 ± 34%, respectively (not significant). Heart rate responses during the hypoxic pulses test did not differ between the first and sixth hypoxic pulse and between control and propofol studies.

DISCUSSION

Influence of Propofol on the Ventilatory Response to Acute Hypoxia

In this study we observed that, in a healthy, young group of male volunteers, propofol, at a sedative concentration (mean BIS value 76), caused a ~50% reduction of the

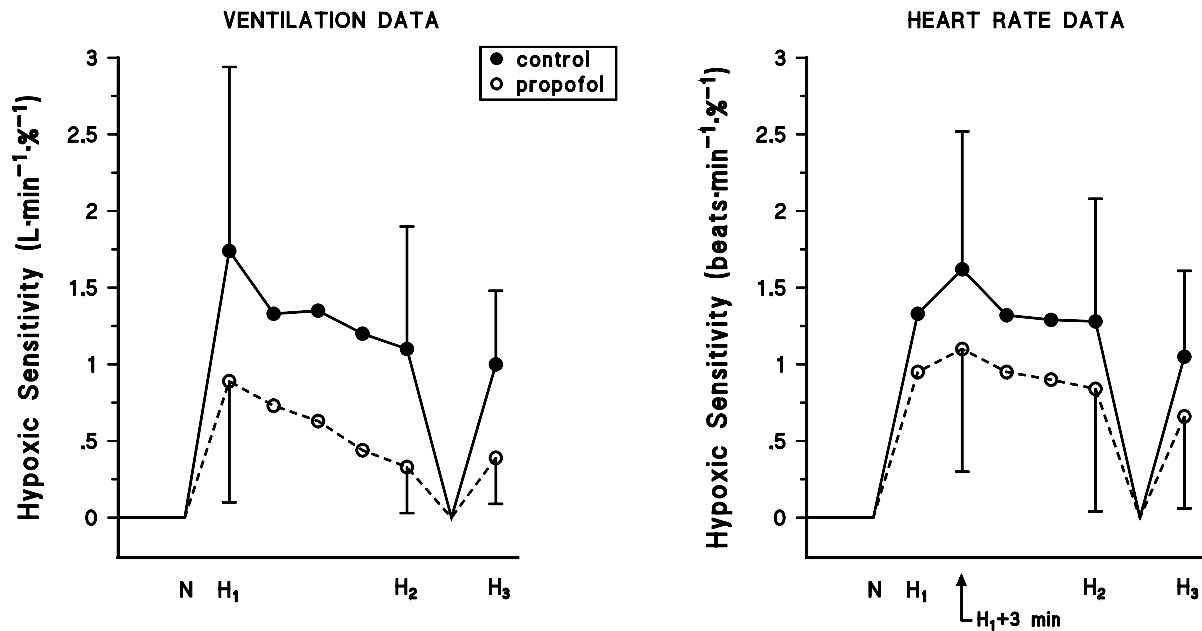


Figure 2. The mean ventilatory (*left*) and heart rate (*right*) responses to the sustained hypoxic test expressed as change in \dot{V}_i or heart rate per percent in arterial hemoglobin-oxygen saturation (S_{pO_2}). For periods N, H₁, H₂, and H₃ see legend of figure 1. The data points between periods H₁ and H₂ are one minutes averages of minutes 6 (i.e., H₁ + 3 min), 9 and 12 of hypoxia. Values are mean \pm SD.

ventilatory response to acute hypoxia. Two previous studies examined the influence of propofol on the hypoxic ventilatory response. Blouin *et al.*²⁰ tested the influence of propofol at a blood concentration of 2 $\mu\text{g}/\text{mL}$ on a ramp hypoxic test. They observed an 80% depression of the ventilatory response in eight male volunteers. However, because the ramp hypoxic test is a mixture of AHR and HVD,¹⁹⁵ their finding is best compared with our observation of propofol-induced reduction of the sustained hypoxic response by about 60%. Nagyova *et al.*¹³⁰ showed a reduction of the AHR by 21, 23 and 60% at respective propofol blood concentrations of 0.5, 1 and 2 $\mu\text{g}/\text{mL}$. This is less than our finding of 50% depression at 0.6 $\mu\text{g}/\text{mL}$. However, in the study by Nagyova *et al.*'s study, differences between the level of consciousness in awake and drug studies were minimized by encouraging the subjects to watch television. There is now ample evidence that this induces behavioral control of breathing. As a consequence additional (nonchemical) drives increase responses mediated *via* the carotid bodies.⁴⁶

Our study gives little information on the site of action of propofol with respect to its effects on normoxic resting ventilation and AHR. Propofol may affect ventilatory control at four possible sites: at the peripheral or central chemoreceptors; at the respiratory centers in the brain stem; at the neuromechanical link between brain stem and ventilation (this includes spinal motoneurons, respiratory muscles, lungs and airways); at sites in the central nervous system involved in behavioral-state control (for example the brain stem reticular system). In cats and rabbits, Ponte and Sadler¹⁵¹ showed that a high dose propofol infusion (18 to 35 mg/kg per h) into the carotid artery abolished chemorecep-

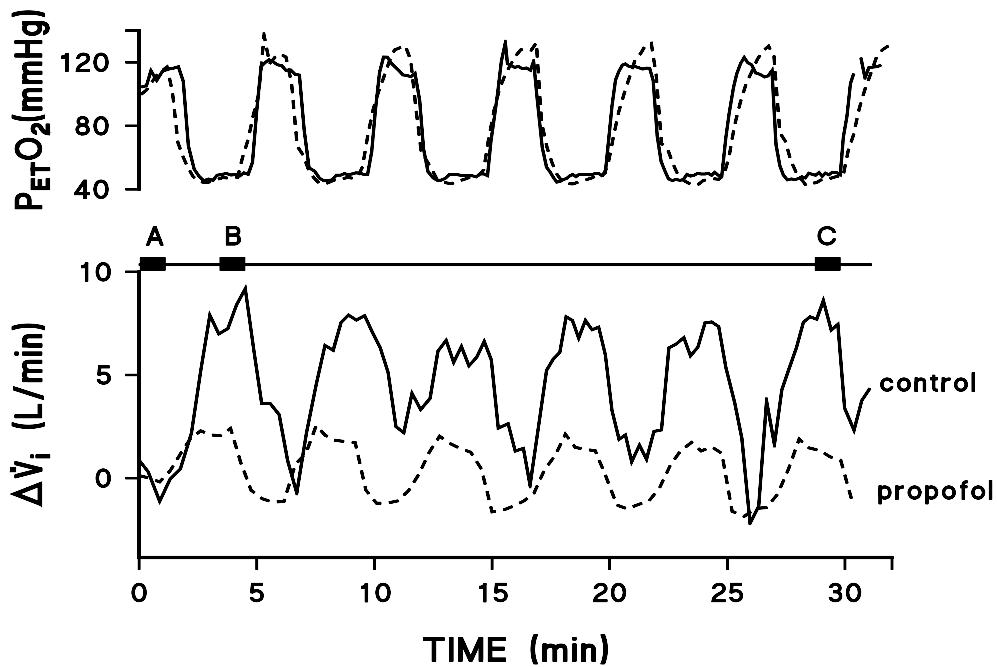


Figure 3. Control and propofol ventilatory responses to intermittent 3-min hypoxic episodes (six hypoxic pulses) of one subject. In between hypoxia there are 2-min normoxic periods. *TOP*. End-tidal pressure of oxygen input to the subject. *BOTTOM*. Ventilatory responses above normoxic baseline (\dot{V}_i data are averaged over six breaths). Period A is the 1-min period before the first hypoxic episode; period B is min 3 of the first hypoxic episode; period C is min 3 of 6th hypoxic episode. Control = continuous line; propofol = dashed lines.

tor discharge in normoxia and prevented an increase during hypoxia. In humans Dow and Goodman⁶² showed that during propofol anesthesia, sudden exposure to hyperoxia reduces \dot{V}_i . This suggests that the peripheral chemoreceptors remain active during propofol anesthesia but does not exclude some depression. In anesthetized cats, an inhibitory effect of propofol on cells in the dorsomedial and ventrolateral medulla mediating pressor effects has been observed.²¹⁵ Both sites are involved in respiratory control and may contain central chemoreceptors.¹⁹⁸ We suggest that, in our study, propofol decreased AHR by depression of the peripheral chemoreflex loop, either directly by enhancing GABAergic inhibition of respiratory centers within the brain stem or indirectly by changing the behavioral state of the subjects. At this time, we are unable to exclude an effect of propofol at the peripheral chemoreceptors.

The EEG data gives some evidence for an effect of propofol on ventilatory control via sites involved in behavioral-state control. We used the BIS of the EEG as measure of the state of sedation/ or hypnosis. Among subjects, we observed a large variability in blood propofol concentrations and BIS values. We relate this to the relatively large interindividual variability in pharmacokinetics and dynamics of intravenous anesthetics. For the BIS, we relate this further to the occurrence, at least in some subjects, of natural sleep on top of drug-induced sedation. Non-rapid eye movement and slow-wave sleep cause further decreases in BIS.¹⁸⁵ In figure 5, we plotted the BIS values of individual subjects (*x*-axis) *versus* the depression of the AHR (*y*-axis, AHR as percentage of con-

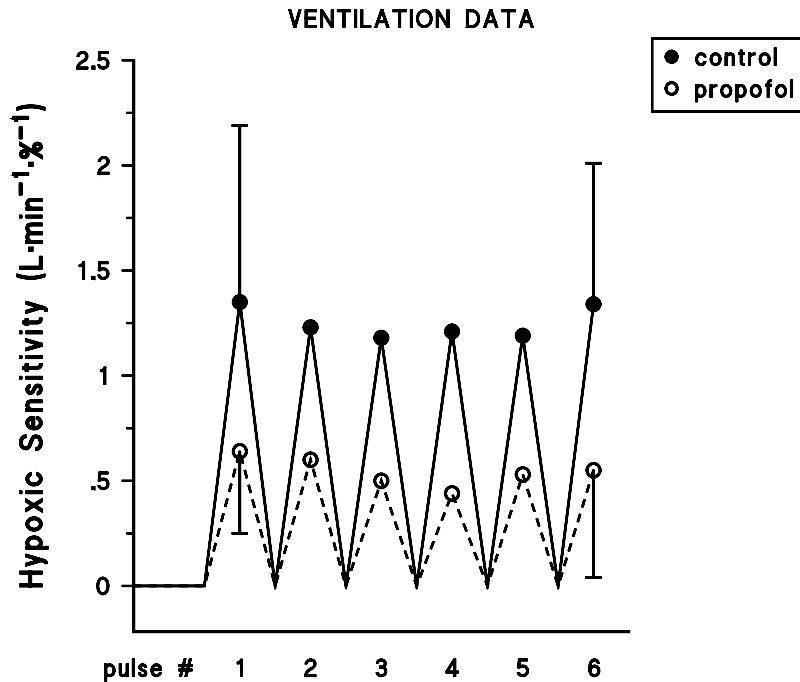


Figure 4. The mean ventilatory responses to hypoxic pulses expressed as change in \dot{V}_i per percentage in arterial hemoglobin oxygen saturation. Hypoxic duration of each pulse is 3-min. For both control and propofol, the response to the last hypoxic pulses (no. 6) is not different from the response to the first pulse. Values are mean \pm SD.

trol and obtained from both hypoxic studies). The data suggest the existence of two (behavioral and respiratory) states in our subjects. Subjects were either awake (control studies [open squares in the right part of figure 5]), and propofol studies with little to no effect on AHR [open circles in the right part of figure 5]) or sedated or asleep with 50 to 60% depression of AHR (closed circles in the right part of figure 5). In the latter group, variations in the level of sedation or hypnosis (as measured by the BIS) had little further effect on AHR.

Influence of Propofol on Hypoxic Ventilatory Decline

In humans, the mechanism of the secondary decline in \dot{V}_i during hypoxia of longer duration (> 3 min) is still under debate. The following observations with respect to HVD are of importance to our study and may provide some insight into the mechanism of HVD:

1. Moderate hypoxia causes an increase in cerebral blood flow (CBF).⁹⁸ Because of the increase in CBF, the carbon dioxide tension within the central nervous system and hence the central ventilatory drive are decreased. As a consequence a significant part of HVD may be related to an increase in CBF.
2. When HVD develops, its influence on \dot{V}_i does not dissipate immediately upon the termination of hypoxia. Subsequent hypoxic responses remain depressed (ratio $AHR_2/AHR_1 < 1$) and one hour of air breathing is necessary for a full recovery

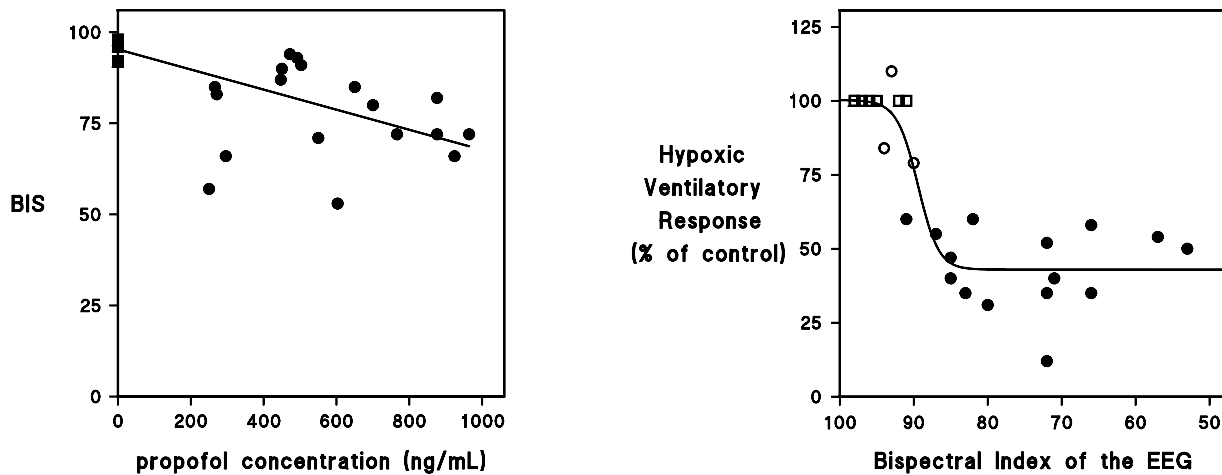


Figure 5. *Left.* The relationship between blood propofol concentration and bispectral index (BIS). Note the twofold larger range in propofol concentration data compared with BIS data. The squares are data points obtained before propofol administration, the circles during propofol infusion. The line through the data is linear regression. *Right.* Influence of the sedative or hypnotic state as measured by the BIS of the electroencephalogram on the depression by propofol of the acute hypoxic ventilatory response (expressed as percentage of awake values). Squares = control data; open circles = propofol data in "awake" subjects as judged by their BIS values and acute hypoxic response. To guide the eye, a sigmoid function (the Hill equation) was fitted to the data. The BIS value at which 50% of the effect occurred is 89.

of AHR ($AHR_2/AHR_1 = 1$).^{50,67} These observations have led to hypothesis that the accumulation or release of neurotransmitters or modulators with net inhibitory influences on \dot{V}_i is quantitatively the more important contributor to HVD. The most important neurotransmitters candidates are GABA and adenosine.^{49,50,67,124,206,210} On the termination of hypoxia, their turnover is not immediate and therefore the depressant effects of hypoxia wane slowly. Animal studies give further proof for a role of central inhibition from GABA. Bicuculline, a GABA_A receptor antagonist, reverses HVD in the anesthetized cat.¹²⁴

3. Among and within subjects, the ratio HVD/AHR_1 is constant with changing values of AHR_1 .^{50,67} This is explained by a modulatory effect of the hypoxic drive of the peripheral chemoreceptors on the central (i.e. within the central nervous system) development of HVD.⁵⁰

In our study, propofol did not affect the absolute magnitude of HVD. However, relative to AHR_1 , HVD did increase, causing an increase in ratio HVD/AHR_1 . Furthermore, the ratio AHR_2/AHR_1 decreased during propofol infusion. Because propofol concentrations and BIS values were constant over time, we argue that these observations indicate that propofol enhanced the development of HVD. Recent studies indicate that propofol acts at GABA_A receptors and enhances GABA-ergic transmission.^{51,107,145} We therefore suggest that the relative increase in HVD is related to propofol's action at the GABA_A receptor complex. Other GABA_A receptor agonists such as midazolam cause similar effects on the ratio HVD/AHR_1 .⁴⁹ Although these observations support a role

for GABAergic neurons in the inhibition of brain stem respiratory centers during sustained hypoxia, they do not exclude the involvement of other receptor systems such as adenosine and opioid receptors. For example, Cartwright *et al.*²⁹ showed an increase of the ratio HVD/AHR₁ by the μ -receptor agonist alfentanil. Aminophylline, an adenosine-receptor blocker, reduces the ratio HVD/AHR₁.⁶⁸ These findings illustrate that HVD development is modulated by various agents commonly used in anesthesia. Studies in humans on the influence of propofol on CBF indicate that propofol reduces CBF concomitant with a reduction in cerebral metabolic rate, and maintains the cerebrovascular response to changes in arterial PCO_2 .⁷³ Although no studies on the influence of propofol on changes in arterial PO_2 are available, it seems improbable that propofol increased the CBF response to hypoxia.

In contrast to 15 min of sustained hypoxia, 15 min of intermittent hypoxia, without and with propofol, were unable to generate HVD. Because in our studies the level of hypoxia was relatively mild (inspired oxygen concentration 10%), a limited number of hypoxic pulses were applied, and the duration of the pulses was relatively short, we are unable to predict the impact of repeated episodes of deeper, longer or more frequent periods of hypoxia on \dot{V}_i . Only limited data are available on ventilatory changes during repeated hypoxia. We recently studied the influence of 0.25% sevoflurane on the ventilatory response to four to six 3-min hypoxic episodes (S_pO_2 84%) separated by 2 min of normoxia in humans.¹⁷⁵ Hypoxic \dot{V}_i remained constant over time. In this respect, the behavior of sevoflurane is identical to propofol. In anesthetized pigs, repetition of 12-min of deep hypoxia (inspired oxygen concentration, 7%) was associated with cardiorespiratory depression during the second hypoxic episode.¹¹⁵ Further animal studies are required to study the interaction of repetitive (deep) hypoxia and anesthetics/hypnotics on \dot{V}_i and hemodynamics.

Influence of Propofol on the Heart Rate Responses to Hypoxia

The Heart Rate response to acute hypoxia depends on the effects of hypoxia at various sites in the body. Decreased heart rate may result from a direct effect of hypoxia on the sinoatrial node or hypoxic stimulation of the carotid bodies resulting in stimulation of the vagal centers in the medulla; increased heart rate may result from activation of aortic chemoreceptors, lung receptors (stimulated *via* an increase in \dot{V}_i), or sites within the central nervous system. These factors interact in a complex fashion.¹¹⁵ In normal breathing subjects, mild to moderate hypoxia is generally associated with tachycardia. Bradycardia is often observed if hypoxia coincides with apnea or at deeper levels of hypoxia.^{53,84}

In both control and propofol studies, the peak heart rate response to hypoxia occurred 6 min after the initiation of hypoxia (figure 2). The reason for the relatively slow dynamics of the heart rate response (compared with the \dot{V}_i response) is unknown but may be related to the complex interaction of the various components responsible for the heart rate response, all with their own dynamics. The results of our study give little evidence for an important role of GABA-mediated hypoxic depression of centers in the brain stem involved in heart rate control. In contrast to the \dot{V}_i data, propofol did not

enhance heart rate decline during sustained hypoxia or increase depression of the heart rate response to a hypoxic episode after 15 min of hypoxia. Furthermore, the decline of heart rate responses in periods H_2 or H_3 (compared with period $H_1 + 3$ min) may be caused by the decrease in \dot{V}_i . Further studies are needed (for example a protocol that allows for a fixed \dot{V}_i level) to elucidate the mechanism by which propofol affects heart rate responses to hypoxia.

It is not appropriate to simply extrapolate our data (obtained in healthy young male subjects, using imposed isohypercapnic hypoxic stimuli [mean S_pO_2 , 86%], without the occurrence of obstructive apnea) to patients under monitored anesthesia care. Studies in patients with sleep apnea indicate that if hypoxia is related to obstructive apnea, initial bradycardia is followed, upon the relief of apnea, by brisk tachycardic responses.^{176,216} In light of the clinical importance of this issue,¹⁶¹ further studies are warranted to examine the influence of sedatives and analgesics on the chronotropic response to hypoxia, especially if upper-airway obstruction is involved.

5 Respiratory sites of action of propofol: *absence of depression of peripheral chemoreflex loop by low dose propofol*

PROPOFOL is frequently used as a monoanesthetic-sedative for various diagnostic or small surgical procedures in patients who breathe spontaneously or is combined with regional anesthesia techniques for larger surgical procedures. Therefore, knowledge on the ventilatory effects of this agent is of importance. In *Chapter 4* we showed that propofol, at a sedative concentration (mean BIS value 76), caused a ~50% reduction of the ventilatory response to acute hypoxia. However the site of action of propofol within the ventilatory control system remains unknown. Propofol may affect breathing at peripheral sites (*e.g.*, peripheral chemoreceptors, lung, diaphragm), central sites (*e.g.*, central chemoreceptors, respiratory centers) or at both sites. All halogenated volatile anesthetics, already at subanesthetic concentrations (0.05–0.2 minimum alveolar concentration (MAC); BIS values ~70–80), cause a selective depression of oxygen (O_2) and carbon dioxide (CO_2) responses mediated by the peripheral chemoreceptors (selective with regard to responses mediated by the central chemoreceptors, which remain unaffected).^{45,47,100,175,202} In this study, we investigated whether propofol has effects on the peripheral CO_2 response similar to those of the inhalational anesthetics. We studied the influence of two concentrations of propofol on the dynamic ventilatory response to hypercapnia in healthy volunteers. Using the dynamic end-tidal CO_2 forcing technique, the ventilatory responses were separated into a fast component originating at the peripheral chemoreceptors and a slow component at the central chemoreceptors.^{12,38} Note that hypoxic studies are unable to resolve the issue of effect-site of a certain agent – anesthetic or analgesic– within the ventilatory control system. The dynamic end-tidal CO_2 forcing technique is especially developed to quantify the contributions of the peripheral and central chemoreflex loops to inspired minute ventilation (\dot{V}_i) in a noninvasive fashion and has been validated extensively in cats and humans.^{12,38,55}

We made two important adaptations in comparison with our earlier studies on the influences of anesthetics and opioids on the dynamic ventilatory response to carbon dioxide. First, to cause a more potent stimulus to the peripheral chemoreceptors, we performed experiments at the background of moderate hypoxia (oxygen saturation 85–90%).³⁸ Second, To increase the precision of the estimation of parameters related to the peripheral chemoreflex loop, we used a multi-frequency binary sequence (MFBS) in end-tidal partial pressure of carbon dioxide (PCO_2) input involving 13 steps into and 13 steps out of hypercapnia.¹⁴⁴

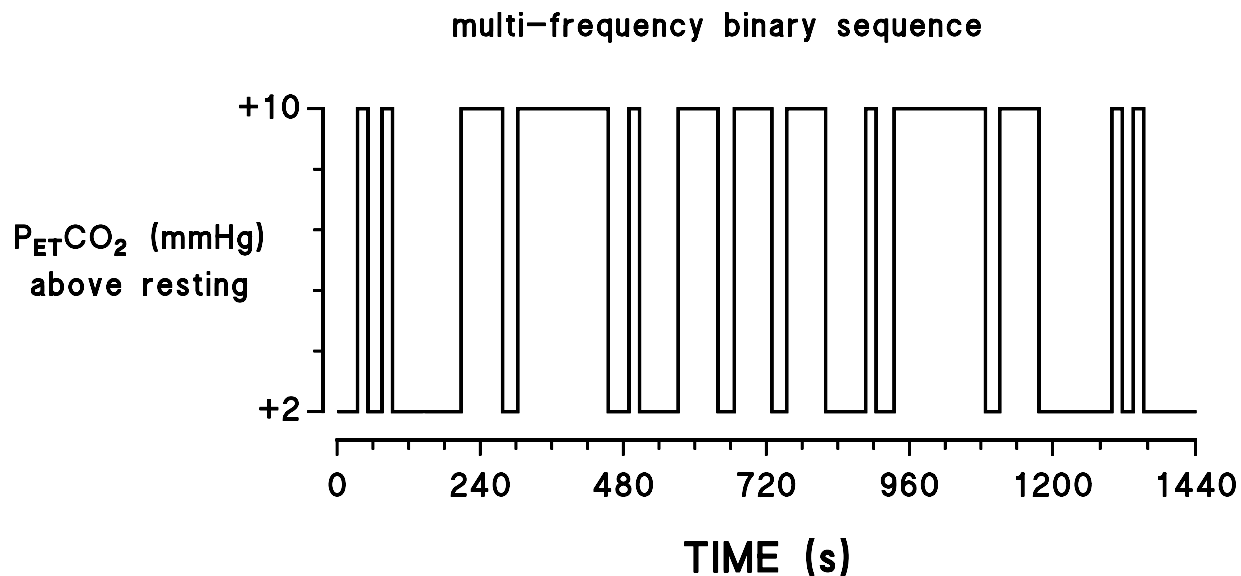


Figure 1. The $P_{ET}CO_2$ multifrequency binary sequence. The y -axis represents the increase in $P_{ET}CO_2$ above the individual subjects' resting $P_{ET}CO_2$ values.

METHODS

Subjects and Apparatus

Ten healthy volunteers aged 18–25 yr (7 men and 3 women) participated in the protocols after approval was obtained from the local Human Ethics Committee. The subjects were healthy and did not have a history of tobacco or illicit drug abuse.

After arrival at the laboratory, two intravenous catheters were inserted in the left and right cubital vein (one for propofol administration and one for blood sampling). Subsequently, electrodes for electroencephalographic measurement (BisSensor; Aspect Medical System, Newton, MA) were placed on the head as specified by the manufacturer, and the subjects rested for 20–30 min. Next a face mask was placed and the studies started.

See METHODS section *Apparatus* of *Chapter 2* for a description of the procedure and apparatus. The electroencephalogram was recorded using an Aspect A-2000 EEG monitor (Aspect Medical Systems; software version 3.3). The BIS values were averaged over 1 min-intervals.

Study Design

The end-tidal PCO_2 was varied according to a MFBS that involved 13 steps into and 13 steps out of fixed $P_{ET}CO_2$ levels (low and high CO_2 : 2 mmHg and 12 mmHg above the subjects' normal air breathing values for $P_{ET}CO_2$) altogether lasting 1408 s (23 min and 28 s). Figure 1 shows a schematic diagram of the $P_{ET}CO_2$ input function. The MFBS experiments were performed at a background of moderate hypoxia ($P_{ET}O_2 = 70$ mmHg) and started 20 min after the initiation of hypoxia. This was done to allow time for hypoxic ventilatory decline to develop prior to investigating the response to CO_2 .

Drug Administration and Sampling

A Psion palm-top computer (London, England) programmed with a three compartment propofol pharmacokinetic data set was used to control a Becton Dickinson infusion pump (St. Etienne, France) for intravenous administration of propofol.⁷⁹ Each subject performed two control MFBS experiments, two during low dose propofol infusion (P_{low} ; target plasma concentration,

0.75 $\mu\text{g/ml}$) and two during high dose propofol infusion (P_{high} , target plasma concentration, 1.5 $\mu\text{g/ml}$). Control studies preceded propofol studies, and low dose propofol experiments preceded high dose propofol experiments. MFBS studies started 15 min after plasma target concentrations had been reached. The duration of propofol infusion was 150 min (75 min for P_{low} and 75 min for P_{high}).

Six venous propofol samples were obtained before and after each of the MFBS experiments. The samples were collected in syringes containing potassium oxalate, and propofol concentrations were determined by reverse-phase high performance liquid chromatography.

Data Analysis

The data were analyzed by fitting the breath-to-breath ventilatory responses to a two-compartment model, as described previously.^{12,38,55,171} The steady state relation of \dot{V}_i to $P_{ET}CO_2$ at constant $P_{ET}O_2$ in humans is described by:

$$(1) \quad \dot{V}_i = (G_p + G_c) \cdot [P_{ET}CO_2 - B_k]$$

where G_p = the carbon dioxide sensitivity of the peripheral chemoreflex loop, G_c = the carbon dioxide sensitivity of the central chemoreflex loop and B_k = the apneic threshold or extrapolated $P_{ET}CO_2$ of the steady state ventilatory response to carbon dioxide at zero \dot{V}_i . The sum of G_p and G_c is the total carbon dioxide sensitivity (G_T). To describe the delay in effect and dynamics of the peripheral and central ventilatory responses to CO_2 , time delays (T) and time constants (τ) are incorporated in the model. The deterministic model parameters are as follows: B_k , G_c , G_p , time constant of the peripheral chemoreflex loop (τ_p), time constant of on-response the central chemoreflex loop, *i.e.*, at high $P_{ET}CO_2$ (τ_{ON}), time constant of the off-response of the central chemoreflex loop, *i.e.*, at low $P_{ET}CO_2$ (τ_{OFF}), time delays of the central and peripheral chemoreflex loops (T_C and T_P), and a linear trend term.³⁸ The noise corrupting the data was modeled through an external pathway with first order dynamics.³⁸ Estimation of the parameters was performed with a one-step prediction error method.¹¹⁶

Sensitivity Analysis

We performed an *a posteriori* sensitivity analysis. Sensitivity analysis enabled us to determine whether the parameter values could be estimated with finite precision from the actual data.^{37,42,212} The analysis was performed by fixing one parameter (*i.e.*, by not allowing it to be estimated) at a time to a series of values (-100% to +100%) around the 'optimum' value (in terms of the 'cost' function or residual sum of squares of the difference between measured and estimated ventilation). The other parameters were estimated by minimizing the residual sum of squares. The shape of the relation between parameter and residual sum of squares informed us of whether parameters were estimable using the specific $P_{ET}CO_2$ and $P_{ET}O_2$ inputs.^{37,42,212} Furthermore, because we performed the sensitivity analysis on actual data (and not on simulated data), we were informed whether local minima existed.

Statistical Analysis

The estimated parameters of control and propofol experiments were subjected to a two-way analysis of variance and *post hoc* least significant differences tests. *P*-values less than 0.05 were considered to be significant. All values reported are mean \pm SD.

Table 1. Estimated Model Parameters, Propofol Concentrations and Bispectral Index (BIS) Values

	control	propofol low dose	anova* vs. control	propofol high dose	anova* vs. control
B_k (mmHg)	36.3 ± 2.7	35.0 ± 2.1	0.009	34.6 ± 1.9	0.002
G_c ($L \cdot \min^{-1} \cdot \text{mmHg}^{-1}$)	1.53 ± 0.36	1.20 ± 0.29	0.009	0.92 ± 0.12	< 0.001
G_p ($L \cdot \min^{-1} \cdot \text{mmHg}^{-1}$)	0.53 ± 0.26	0.47 ± 0.19	ns	0.46 ± 0.19	ns
G_T ($L \cdot \min^{-1} \cdot \text{mmHg}^{-1}$)	2.07 ± 0.50	1.67 ± 0.43	0.006	1.42 ± 0.60	< 0.001
G_p/G_T	0.26 ± 0.08	0.28 ± 0.06	ns	0.33 ± 0.06	ns
Trend (ml/min per min)	110 ± 66	39 ± 61	ns	20 ± 70	0.02
$C_{\text{propofol } A}$ ($\mu\text{g/ml}$)		0.44 ± 0.13		1.18 ± 0.30	
95% c.i.		0.32 — 0.53		0.95 — 1.41	
$C_{\text{propofol } B}$ ($\mu\text{g/ml}$)		0.54 ± 0.12		1.27 ± 0.32	
95% c.i.		0.45 — 0.64		0.97 — 1.57	
$C_{\text{propofol } C}$ ($\mu\text{g/ml}$)		0.49 ± 0.09		1.36 ± 0.22	-
95% c.i.		0.42 — 0.57		1.18 — 1.55	
BIS	97 ± 2	84 ± 8	< 0.001	67 ± 14	< 0.001

Values are mean ± SD. There were no time effects on the propofol concentrations (analysis of variance, anova).
* *post hoc* least significance test vs. control.

G_c = central carbon dioxide sensitivity; G_p = peripheral carbon dioxide sensitivity;

G_T = total carbon dioxide sensitivity; ns = nonsignificant; CI = confidence interval;

A, B, C = samples before the first multifrequency binary sequence, between multifrequency binary sequences, and after the second multifrequency binary sequence.

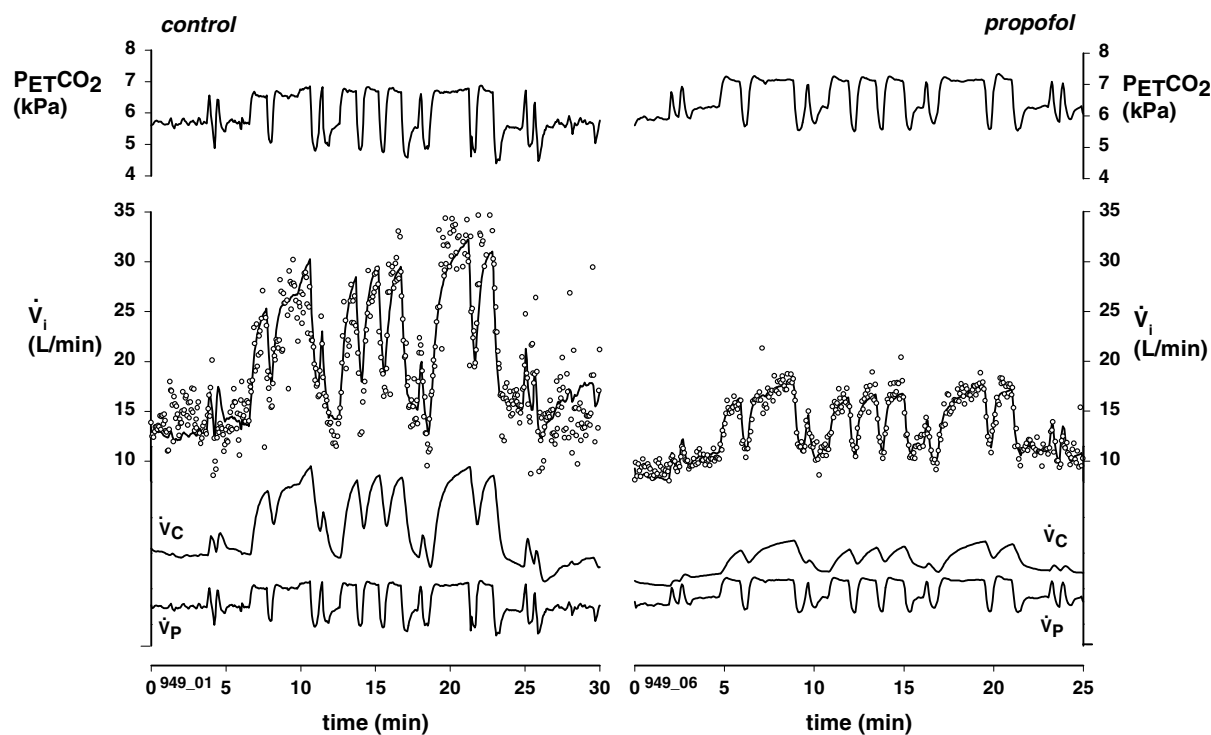


Figure 2. Control, left, and propofol, right, ventilatory responses to carbon dioxide of one subject. TOP: $P_{ET}CO_2$ input function is shown and is varied according to a multi-frequency binary sequence that involved 13 steps into and 13 steps out of hypercapnia. BOTTOM: Each circle represents one breath. The thick line through the breaths is the deterministic part of the model, which is the sum of the outputs of the peripheral (\dot{V}_p) and central (\dot{V}_c) chemoreflex loops and a trend term. Estimated control parameter values are as follows: B_k , 36.8 mmHg, G_p , 0.40 L·min⁻¹·mmHg⁻¹, and G_c , 1.61 L·min⁻¹·mmHg⁻¹. Estimated propofol parameter values are as follows: B_k , 36.5 mmHg, G_p , 0.35 L·min⁻¹·mmHg⁻¹ and G_c , 0.75 L·min⁻¹·mmHg⁻¹.

RESULTS

All subjects terminated the protocol without side effects. Because of propofol, the arousal state of the subjects decreased with Bispectral Index values of 84 ± 8 at low dose propofol infusion and 67 ± 14 at high dose propofol infusion. The concentration of propofol remained constant over time during the two infusion schemes (table 1). Examples of a control and a propofol MFBS experiment (propofol target = 1.5 μ g/ml) and model fits of one subject are given in figure 2. Only the deterministic part of the model is shown. It shows a large effect of propofol on the output of the central chemoreflex loop (a 55% reduction of G_c) with only a minor effect on the output of the peripheral chemoreflex loop (a 12% reduction of G_p).

The averaged model parameters are collected in table 1. At all three treatment levels, the estimated model parameters did not differ between the first and second CO_2 response. Propofol reduced the total CO_2 sensitivity (G_T) by approximately 20% at a propofol target of 0.75 μ g/ml and by approximately 34% at a target of 1.5 μ g/ml. At

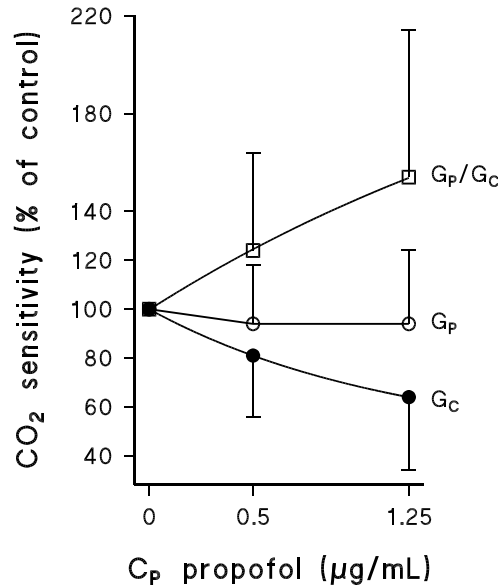


Figure 3. Influence of propofol on the ventilatory carbon dioxide (CO_2) sensitivities relative to control values for peripheral CO_2 sensitivity (G_p), central CO_2 sensitivity (G_c) and total CO_2 sensitivity (G_T). values are mean \pm SD.

both propofol concentrations, the reduction of G_T was caused by a reduction of the output of the central chemoreflex loop by 20% and 40% at low and high dose propofol, respectively, without affecting the output of the peripheral chemoreflex loop. As a consequence the ratio G_p to G_c is increased relative to the control state (figure 3). The apneic threshold (B) showed a small but significant reduction during propofol infusion (table 1). The time constants and time delays of both chemoreflex loops remained unaffected by propofol (data not shown).

Figure 4 shows the results of the sensitivity analysis of the model parameters in one subject. A well defined minimum of the residual sum of squares was observed for all parameters, indicating that they could be identified with acceptable accuracy (including parameters T_p and T_p , not shown). The most accurately estimated parameters were B_k and G_p , as shown by the steepness of the increase in residual sum of squares at parameter values above and below the optimum. The shape of the curves for G_c , τ_p , τ_{ON} , and τ_{OFF} are markedly asymmetric, indicating that the estimation may be less accurate at values higher than the optimum. As expected, the steepness of the increase in residual sum of squares of G_p is less when this parameter is estimated from single step CO_2 input function (broken line in fig. 4). This indicates that G_p is estimated with greater accuracy from an MFBS input function relative to a single step input. G_c is well estimated from an MFBS and step input. However, the analysis indicates somewhat greater accuracy using a single CO_2 step for values above its optimum and an MFBS input for values below its optimum.

DISCUSSION

We used a multifrequency binary sequence in $P_{ET}CO_2$ to quantify the effect of propofol on ventilatory control. The MFBS was designed by Pedersen *et al.*¹⁴⁴ to spread its power over the frequency range of interest for identification of both peripheral and central chemoreflex responses and to optimize identification of the peripheral chemoreflex response. Using a single step, the peripheral response is determined from only a limited portion of the data (2 min of the 15–20 min of a CO_2 study). Using an MFBS, this increases significantly (19.5 min of a 24 min experiment). Consequently, the precision of estimation parameters related to the peripheral chemoreflex loop is greater when derived from MFBS compared with single steps. Indeed, our sensitivity analysis indicates the improvement of the estimation of the peripheral CO_2 sensitivity compared with a step $P_{ET}CO_2$ function without compromising the accuracy of estimation of central CO_2 sensitivity (fig. 4).

We used a target-controlled infusion system to administer propofol. Fifteen minutes after target plasma concentrations of propofol were attained, the respiratory studies started. Estimation of the effect-site propofol concentration indicated that this time was ample for equilibrium between blood and effect-site. We measured venous propofol concentrations, which may not reflect arterial or effect-site concentrations. However, we observed no time effect on venous propofol concentrations or on parameter estimates at P_{low} or P_{high} . This indicates stable arterial and effect-site propofol concentrations and suggests a small gradient between venous and arterial propofol concentrations.

With respect to the control of breathing propofol may have an effect at the central or peripheral chemoreceptors, at the respiratory centers in the brainstem, at the neuromechanical link between brainstem and ventilation (\dot{V}_i), or at sites in the central nervous system involved in behavioral state control. The exact location of the central chemoreceptors is unknown but they are probably located in the dorsomedial medulla, the rostroventrolateral medulla, or both.¹⁹⁸ The peripheral chemoreceptors are located in the carotid bodies, which are strategically situated at the bifurcation of the common carotid arteries and have an important role in oxygen delivery to the brain. The peripheral chemoreceptors respond to changes in arterial oxygen tension (PaO_2) and arterial carbon dioxide tension ($PaCO_2$).^{14,15}

We observed that propofol, at doses causing a decrease in Bispectral Index to approximately 70, has an important effect on the control of breathing. Specifically, propofol reduced G_c but had little influence on G_p . This indicates the absence of a (selective) effect of low-dose propofol on the peripheral chemoreflex loop. In this respect, propofol stands in sharp contrast to the modern volatile halogenated anesthetics.^{45,50,175,202} Our findings are in agreement with studies from the literature. Dow and Goodman showed in humans that during propofol anesthesia, the carotid bodies retain their ability to respond to hyperoxia.⁶² In anesthetized cats, propofol displayed an inhibitory effect on areas of the dorsomedial and ventrolateral medulla, which possibly contain the central

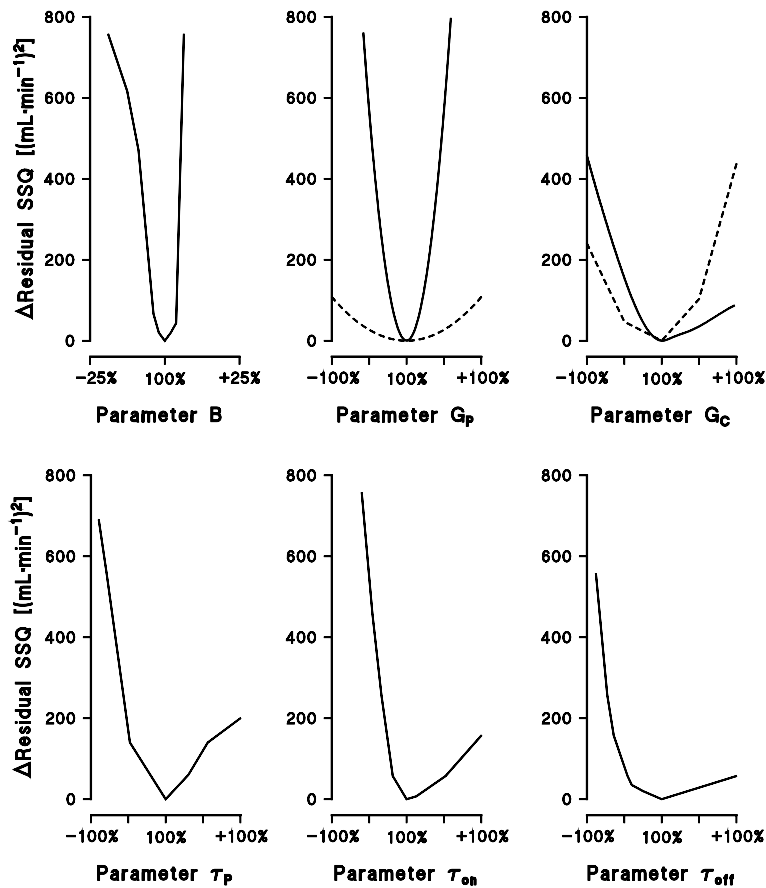


Figure 4. Results of the sensitivity analysis for the model parameters of the empirical carbon dioxide model of the ventilatory controller in one subject. The data are control data obtained using a multifrequency binary sequence input function (continuous lines). For comparative reasons, we added the sensitivity analysis on G_p and G_c obtained from a single step input function of the same subject (subject 936, broken lines). The x -axis gives the optimal parameter value (100%) \pm 100%; the y -axis gives the increase in residual sum of squares (Δ residual SSQ) from the optimal value (residual SSQ set at 0).

chemoreceptors.^{198,215} Evidently, our data do not preclude some depressant effect of higher doses of propofol than used by us on the carotid bodies or its afferent pathways. For example, animal data show that high dose propofol infusion, 18–35 mg/kg per h, causes the cessation of carotid body chemoreceptor activity.¹⁵⁰

Effect of Propofol on Peripheral CO₂ versus O₂ Responses

Our finding of the absence of an effect of propofol up to plasma concentrations of 1.25 μ g/mL on the peripheral CO₂ response seems in disagreement with our observation in *Chapter 4* of a 50% depression of the acute hypoxic \dot{V}_i response by 0.6 μ g/mL propofol. Because the acute hypoxic ventilatory response originates at the peripheral chemoreceptors of the carotid bodies,²⁰⁶ some depression of the peripheral CO₂ response was anticipated from our earlier results. Apart from the possibility that O₂- and CO₂-sensing at the carotid bodies are differentially affected by propofol, there are three conceivable explanations for this discrepancy.

1. Carotid body depression by anesthetics is dependent on stimulus intensity. For example, Ponte and Sadler showed that relative to a mild hypoxic stimulus, a more intense stimulus ($PO_2 < 40$ mmHg) is able to overcome volatile anesthetic-induced depression of the carotid bodies.¹⁵¹ In analogy, the stimulus in this study (a hypercapnic-hypoxic stimulus of $P_{ET}CO_2$ 13 mmHg above resting and S_pO_2 88–90%) may offset depression of the carotid bodies by propofol as observed previously using a less intense hypoxic stimulus (a hypercapnic-hypoxic stimulus of $P_{ET}CO_2$ 5 mmHg above resting and $S_pO_2 \sim 87\%$). Interestingly, when assessing the effect of low dose volatile anesthetics on ventilatory control, we observed depression of carotid body mediated responses even when intense stimuli, such as used in this study, were applied.^{45,175} This suggests a difference in stimulus intensity needed to overcome carotid body depression caused by propofol and volatile anesthetics.
2. In cats, Berkenbosch *et al.* studied the peripheral \dot{V}_i response dynamics to hypoxic stimulation while the PaO_2 of the medulla oblongata was kept constant using the technique of artificial brainstem perfusion.¹⁵ Mathematically, the responses were best described by two components: a fast component with a time constant of approximately 2 s and a slow component with a time constant of approximately 73 s. The fast component was considered to originate at the carotid bodies, whereas it was argued that the slow component was due to central modulation of the carotid body response (*i.e.*, neuronal dynamics).¹⁵ Interestingly, in the same animal preparation, the response of the peripheral chemoreflex pathway to changes in end-tidal PCO_2 does not show a slow component.¹⁵ This indicates that although peripheral hypoxic stimulation activates central neuronal dynamics, peripheral hypercapnic stimulation does not. Also, in humans, the hyperventilatory response to hypoxia is well-described by a fast and a slow component.⁹⁷ In *Chapter 4* we studied the effect of $0.6 \mu\text{g/ml}$ propofol on the ventilatory responses to 3 min hypoxic pulses, we reanalyzed the data using the two-component model as described by Berkenbosch *et al.*¹⁵ All control curves were best described by two components as judged by the Akaike criterion,¹ with time constants of 3 and 100 s for the fast and slow components, respectively. Propofol did not affect the gain (*i.e.*, hypoxic sensitivity) of the fast component (ratio $G_{propofol}/G_{control} = 0.95$), but caused a significant reduction of the gain of the slow component (ratio $G_{propofol}/G_{control} = 0.45$, $P < 0.05$). If we assume that the fast response reflects the carotid body response to hypoxia and the slow component central neuronal dynamics,^{15,39} these results suggest that propofol affects central neuronal dynamics but has little effect on the carotid bodies or their output, and thus does not reduce G_p . This is in contrast to the effect of inhalational anesthetics. We previously studied the effect of sevoflurane, 0.25% end-tidal (~ 0.15 MAC), on the ventilatory responses to 3 min hypoxic pulses,¹⁷⁵ and reanalyzed the data using the two-component model as described above. Sevoflurane reduced the fast and slow component by 25 and 60%, respectively ($P < 0.05$), an indication for an effect of sevoflurane on the carotid bodies and on central neuronal dynamics.

3. Apart from a stimulatory effect at the carotid bodies, hypoxia causes depression of ventilation *via* central mechanisms, *i.e.*, within the central nervous system.⁴⁸ The central effect of hypoxia on \dot{V}_i is already apparent after 1 min of hypoxic exposure,³⁹ therefore, any measured hypoxic \dot{V}_i response is the mixture of carotid body and central effects on \dot{V}_i . Because propofol enhances the magnitude of the central depressant effects of hypoxia in humans,* greater depression by propofol of the measured ventilatory response to hypoxia relative to the measured peripheral CO_2 response is expected.

These three mechanisms should be taken into account when comparing our current results on the effect of propofol on the peripheral and central chemoreflex loops with our results of *Chapter 4* and studies from the literature on the effect of propofol on the ventilatory response to acute,¹³⁰ and subacute hypoxia.²⁰

Influence of Propofol versus Inhalational Anesthetics on Peripheral CO_2 Response

The discrepant effects of propofol and sevoflurane on the carotid body response to CO_2 is striking and may be explained by differences in molecular sites of action of intravenous and halogenated inhalational anesthetics. We believe that propofol, like inhalational anesthetics, affects breathing through enhancement of γ -aminobutyric acid-mediated transmission and reduction of glutamatergic activity in the brainstem.¹⁹⁰ This may have induced the depression of G_c in our study. Furthermore, inhalational anesthetics activate background K^+ channels in the peripheral and central nervous system.^{25,143,184} These channels are involved in tonic inhibition of cellular excitability, and activation by volatile anesthetics may be the cause of some major side effects such as depression of cardiac function and respiratory depression. Buckler *et al.* recently showed the existence of an oxygen-, acid- and inhalational anesthetic (halothane)-sensitive background K^+ channel in the carotid body chemoreceptor cells,²⁵ which possibly is an important link in the cascade leading to CO_2 - and O_2 -sensing in the carotid bodies and the selective site of inhalational anesthetic depression of carotid body function[†]. Further studies are needed to show how anesthetics (including propofol) modulate the pH- PO_2 sensitivity of these background channels.

*see *chapter 4*

†see also *Chapter 3*

6 Response Surface Modeling of Alfentanil–Sevoflurane Interaction on Cardiorespiratory Control and Bispectral Index

ONE OF THE ADVANTAGES of combining an opioid and an anesthetic over the use of single agents is the synergistic increase in desired anesthetic effect, such as absence of movement in response to a painful stimulus as defined by the minimal alveolar concentration (*i.e.*, anesthetic potency).¹¹⁰ The consequence of this mechanism is the need for less drugs with possibly less side effects. Anesthesiologists make use of these fortuitous interactions by combining opioids and anesthetics during anesthesia. Because respiratory depression is a serious side effect of anesthetics,^{47,48} hypnotics,⁴⁹ and opioids,^{43,171} even at low doses, it is surprising that few studies in humans addressed the issue of the impact of drug combinations on respiration. Especially, the nature of the interaction (additive *versus* synergistic) of an anesthetic–opioid combination on the control of breathing remains unknown. Therefore, we studied the influence of the opioid alfentanil and the inhalational anesthetic sevoflurane on ventilatory control, heart rate (HR), bispectral index (BIS), as measure of the hypnotic state of the subjects, and the ventilatory and HR responses to hypoxia. The ability of the ventilatory control system to cope adequately with hypoxic episodes is of importance because hypoxic periods frequently occur perioperatively.

The alfentanil–sevoflurane interaction on ventilatory control, HR and BIS was assessed by response surface modeling.^{82,191,129} This approach enables us to construct three dimensional representations of the concentration–response relation among combinations of alfentanil and sevoflurane and assess the nature of the interaction (additive, synergistic or antagonistic) over the whole surface area (it is possible for the response surface to include all of these interactions in different regions).^{82,191,129} This approach is superior to the construction of isoboles (or iso-effect curves), which allows assessment of the interaction at drug combinations yielding a constant effect, such as 25% or 50% reduction in effect parameter (*i.e.*, C_{25} and C_{50}).

In this study we used the dynamic end-tidal forcing technique.³⁸ This technique enables us to assess the effect of drug combinations on ventilatory control at identical end-tidal partial pressures of carbon dioxide ($P_{ET}CO_2$'s). Consequently, this makes a comparison among the respiratory effects of different drug combinations possible because the results are not confounded by changes in $P_{ET}CO_2$.

METHODS

Subjects and Apparatus

Nine healthy male volunteers (aged 18–25 yr) participated in the protocol after approval was obtained from the local human ethics committee (Commissie Medische Ethiek, Leiden Univer-

Table 1. Total Number of Paired Hypoxic Studies at Each of the Treatment Concentrations

		Target Alfentanil Conc. (ng/ml)					
		0	10	20	30	40	50
ET sevoflurane (%)	0	18	4	9	3	9	2
	0.05	1	1	2	2	-	-
	0.1	9	5	9	2	5	-
	0.2	9	3	7	2	1	-
	0.3	9	3	3	-	-	-
	0.4	2	-	-	-	-	-

sity Medical Center, 2300 RC Leiden, The Netherlands). Oral and written consent was obtained from all volunteers. The subjects were healthy and did not have a history of tobacco or illicit drug use.

After arrival at the laboratory, two intravenous catheters were inserted in the left and right antecubital vein (one for alfentanil administration and one for blood sampling). Subsequently electrodes for electroencephalographic measurement were placed on the head as specified by the manufacturer and the subjects rested for 20–30 min. Next, a face mask was applied over the mouth and nose and the experiments started.

See METHODS section *Apparatus* of Chapter 2 for a description of the procedure and apparatus. The electroencephalograph was recorded using a monitor that computed the BIS over 2-s epochs. We averaged the BIS values over 1 min-intervals and used data points obtained at 3-min intervals.

Study Design

The ventilatory response to isocapnic hypoxia (acute hypoxic response) was assessed during inhalation of sevoflurane, infusion of alfentanil and the combined administration of both agents. Initially, two hypoxic control studies (*i.e.*, studies without the administration of any agent) were obtained. Next inhalation of sevoflurane was started, and hypoxic studies were performed during inhalation of sevoflurane. To achieve blood-brain equilibrium, sevoflurane hypoxic experiments were preceded by a 12-min equilibration period.¹³⁷ In table 1, the various imposed target end-tidal sevoflurane concentrations are given. After this set of studies, the subject rested for 30–45 min, and another two control studies were obtained. Next, intravenous infusion of alfentanil was started, and hypoxic studies were performed at various blood target concentrations (table 1). Subsequently, hypoxic studies during the combined administration of sevoflurane and alfentanil were performed (table 1). The sevoflurane and alfentanil target concentrations were chosen in such a way that at least 60% depression but preferably 80% depression, of the ventilatory response to hypoxia was achieved. All experiments were performed on a single day, starting at 08:30 AM.

The Isocapnic Hypoxic Study

The $P_{ET}O_2$ waveform was as follows: (1) 10 min at 110 mmHg; (2) a rapid decrease to 50 mmHg; (3) 3 min at 50 mmHg; and (4) 4–5 min at 110 mmHg. At each treatment level (control, various concentrations of sevoflurane, alfentanil, and sevoflurane plus alfentanil), two hypoxic studies

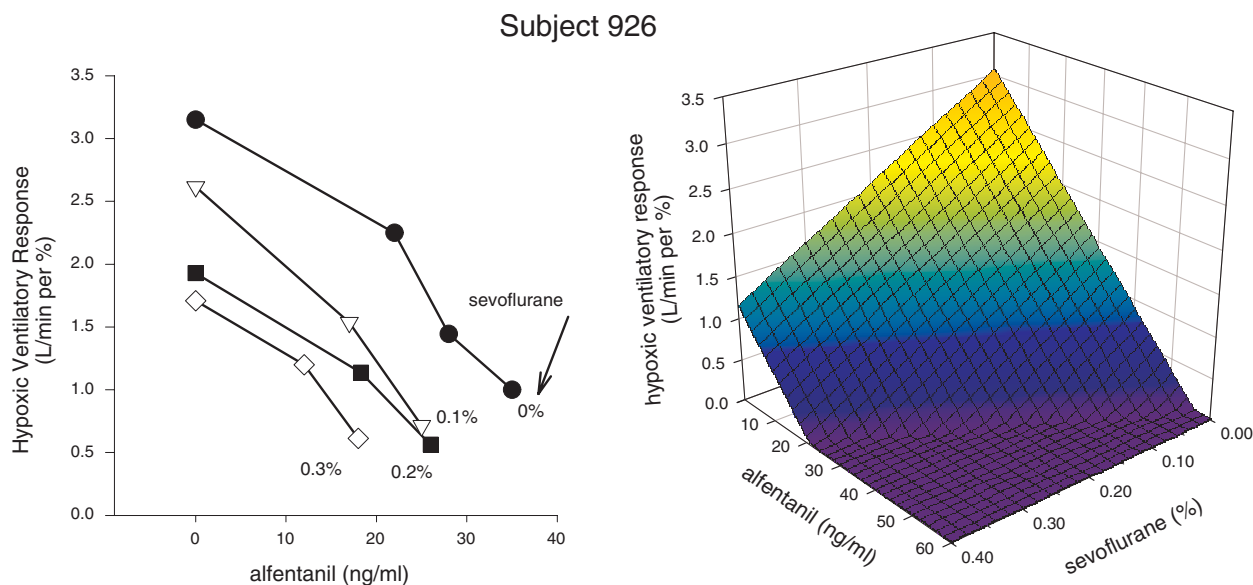


Figure 1. *LEFT.* The influence of plasma alfentanil concentration (x -axis) at fixed concentrations of end-tidal sevoflurane concentration on the hypoxic ventilatory response (y -axis) in one subject (subject 926). Note the parallel displacements of the alfentanil dose–hypoxic response curve caused by sevoflurane. *RIGHT.* A three dimensional graph of the individual Bayesian estimate of the response surface of subject 926 for the hypoxic ventilatory response. The surface gives an impression of the nature of the interaction at all possible drug combinations, which in this case is additive.

were obtained.

All hypoxic studies were performed at identical end-tidal PCO_2 concentrations, ~ 5 – 7 mmHg more than awake resting values. This high value was chosen to offset an increase in end-tidal PCO_2 caused by the alfentanil-sevoflurane-induced ventilatory depression.

Alfentanil Administration, Blood Sampling, and Assay.

A target controlled infusion was used for the administration of alfentanil. A palm-top computer programmed with the population pharmacokinetic data set, reported by Maitre *et al.*¹¹⁹ was connected to a syringe pump, which was filled with alfentanil (0.5 mg/ml). This system allows a theoretical plasma concentration of alfentanil to be achieved rapidly and maintained. Hypoxic studies were performed 5–10 min after blood alfentanil concentrations reached their target levels. Because this equals 5–10 times the alfentanil blood-brain equilibration half-life of 1 min,¹⁷⁹ we assume that brain and blood alfentanil concentrations were in equilibrium.

Before and after changes in target alfentanil concentration, 3 ml blood samples for the measurement of alfentanil were collected. A capillary gas chromatographic technique was used to determine the plasma concentration.

Data Analysis and Statistics

Averaged values of the breath-to-breath data were chosen over identical time segments. Normoxic data points were the mean of the 10 breaths before the 3-min hypoxic period. Hypoxic data points were mean of the last 10 breaths of the hypoxic episode. Analysis was performed on the following variables: normoxic \dot{V}_i , normoxic V_T , normoxic RR, normoxic HR, normoxic BIS, normoxic and hypoxic end-tidal PCO_2 , normoxic and hypoxic end-tidal partial

pressure of oxygen (PO_2); and the change in \dot{V}_i , V_T , RR and HR from normoxia to hypoxia relative to the absolute changes in arterial oxygen saturation (*i.e.*, $\Delta\dot{V}_i/\Delta S_{pO_2}$, $\Delta V_T/\Delta S_{pO_2}$, $\Delta RR/\Delta S_{pO_2}$ and $\Delta HR/\Delta S_{pO_2}$). Hypoxic sensitivities usually are defined as follows: $[E(\text{hypoxia}) - E(\text{normoxia})]/\Delta S_{pO_2}$, where ΔS_{pO_2} is $[100 - S_{pO_2}(\text{hypoxia})]$, and E the value of a measured parameter. Instead of 100, we used the actual S_{pO_2} -value measured during normoxia to calculate ΔS_{pO_2} . This definition results in positive hypoxic sensitivity values when the measured parameter E increases upon the introduction of hypoxia (*i.e.*, $E(\text{hypoxia}) > E(\text{normoxia})$). Only when the effect parameter decreases because of hypoxia (*i.e.*, $E(\text{hypoxia}) < E(\text{normoxia})$), will the value of the hypoxic sensitivity be negative. The data sets (sevoflurane concentration, alfentanil concentration, E parameter) were analyzed using a pharmacodynamic model which can be visualized in three-dimensional space as a 'response surface'. We used the following pharmacodynamic model:

$$(1) \quad f(x) = \alpha \cdot (1 - x^y)$$

By substituting E_0 , which is the baseline value (*i.e.*, pre-drug administration value) of a variable, for α and substituting $U^y/2$ for x^y and $U = C/C_{50}$, where C_{50} is the concentration causing 50% decrease of E , E a measured variable and C the concentration of one drug we obtain:

$$(2) \quad E(C) = E_0 \cdot (1 - U^y/2)$$

Pure additive interactions of two drugs (alfentanil and sevoflurane) are modeled as follows:

$$(3) \quad E(C_A, C_S) = E_0 \cdot (1 - (U_A + U_S)^y/2)$$

$$(4) \quad U_A = C_A/C_{50,A}$$

$$(5) \quad U_S = C_S/C_{50,S}$$

C_A is the alfentanil plasma concentration, C_S the end-tidal sevoflurane concentration, $C_{50,A}$ is the alfentanil concentration causing a 50% decrease of E , $C_{50,S}$ is the sevoflurane concentration causing a 50% decrease of E .

To include non-additive interactions in the model, an interaction, term $I(Q)$, is introduced:

$$(6) \quad E(C_A, C_S) = E_0 \cdot (1 - [(U_A + U_S)^y]/2 \cdot I(Q))$$

$I(Q)$ is a smooth function (spline) of Q (see Appendix 1). Following Minto *et al.*,¹²⁹

$$(7) \quad Q = U_A/(U_A + U_S)$$

Q ranges from 0 (sevoflurane only) to 1 (alfentanil only) and is the drug concentration ratio of alfentanil and sevoflurane normalized by their respective C_{50} s or potencies (equations 4 and 5). The smooth function has two parameters, I_{max} and Q_{max} . I_{max} is the maximum value of the interaction term, and Q_{max} is the value of Q (*i.e.*, concentration ratio) for which I attains I_{max} . When I_{max} equals 1, the interaction is purely additive. An I_{max} of less than 1 denotes antagonism and an I_{max} of more than 1 denotes synergy.

The model was fitted to the data with NONMEM (conditional estimation method), version V, level 1.1 (a data analysis program for non-linear mixed effects modeling)* using a population approach. The interindividual variability of each of the model parameters is characterized by the percentage coefficient of variation, which is a parameter derived from the variance of the logarithm of the individual model parameters. Likelihood-ratio tests were performed to determine whether γ did not equal 1 and whether I_{max} did not equal 1. The intraindividual variability was quantified by the standard deviation of the residuals. P -values of less than 0.01 were considered significant.

RESULTS

A total of 109 paired hypoxic responses were obtained at various treatment levels (table 1). The duration of the experiments ranged from 5 to 6 hours. In figure 1, an example of the various drug combinations applied in a single subject is shown. This graph further illustrates that the maximum depression of the hypoxic drive ($= \Delta\dot{V}_i/\Delta S_{pO_2}$) was 70 to 80%, obtained at multiple drug combinations. This level of depression was one of the aims of the study protocol, which was met in all subjects.

End-tidal carbon dioxide values were equal in normoxia and hypoxia ($P_{ET}CO_2$ normoxia 48.3 ± 0.5 mmHg (mean \pm SEM) versus hypoxia 48.2 ± 0.6 mmHg). These values are 6–7 mmHg more than individual resting values. The measured plasma alfentanil values averaged to 12.7 ± 0.9 ng/ml, 21.3 ± 1.6 ng/ml, 27.4 ± 1.2 ng/ml and 35.6 ± 2.4 ng/ml for target concentrations of 10, 20, 30 and 40 ng/ml, respectively.

A three dimensional graph of the conditional Bayesian estimate of the response surface of one subject for $\Delta\dot{V}_i/\Delta S_{pO_2}$ is given in figure 1 (right). The value of γ was not different from 1. This indicates a linear relation between alfentanil, sevoflurane, and effect. The surface gives an impression of the nature of the interaction at all possible drug combinations, at least within the dose ranges tested, which, in this case, is additive in all regions of that part of the surface. It further predicts the interaction at concentrations higher than those we applied. Assuming that the concentration-effect relation remains linear, the model predicts the complete loss of the hypoxic response (i.e., $\Delta\dot{V}_i/\Delta S_{pO_2} = 0$) in this subject at combinations of alfentanil and sevoflurane on a line on the surface connecting the points (alfentanil = 55 ng/ml, sevoflurane = 0 ET%) and (alfentanil = 22 ng/ml, sevoflurane = 0.4 ET%).

The population estimates of the response surfaces are given in table 2 and figures 2–4. The model fit the data well. For all variables, the model fits yielded values of γ not significantly different from 1. This indicates that alfentanil and sevoflurane, and combinations of alfentanil and sevoflurane at fixed ratios, cause changes of the measured variables in a linear manner. The C_{25} and C_{50} values (the concentrations causing 25% and 50% reduction of effect) are given in table 2. Note the absence of effect of alfentanil on BIS (table 2 and fig. 4) and of sevoflurane on normoxic breathing frequency (table 2;

*NONMEM User's Guides, 1999, SL Beal & LB Sheiner (Eds.) NONMEM Project Group, University of California at San Francisco, San Francisco.

see also below).

Pure additive interactions were found for the following parameters: $\Delta\dot{V}_i/\Delta S_pO_2$, V_T , $\Delta V_T/\Delta S_pO_2$, $\Delta RR/\Delta S_pO_2$, $\Delta HR/\Delta S_pO_2$. Inert interactions were observed for RR and BIS. This leads to straight isoboles or iso-effect curves (the isoboles are shown in figs. 2–4 *Right*). Alfentanil, over the dose range studied, caused a modest reduction of normoxic breathing frequency. The C_{25} was 81.5 ng/ml, clearly outside the dose range we studied (table 2). Sevoflurane had no effect on RR when increasing from 0 to 0.4 ET%. Sevoflurane caused a linear reduction of BIS with an EC_{25} of 0.45 ET% (table 2). The effect of sevoflurane on the BIS was independent of the alfentanil concentration.

Synergistic interactions were found for normoxic \dot{V}_i and HR (table 2 and figs. 2 and 3). In figure 5, the relations between Q (*i.e.*, the alfentanil–sevoflurane concentration ratio) and $I(Q)$ (*i.e.*, the interaction) are given. For \dot{V}_i and HR, synergistic interactions were observed, with maximum synergistic interactions (I_{\max} in table 2) occurring at Q values of approximately 0.7 (Q_{\max} in table 2). Depression of \dot{V}_i by 25% occurred at an alfentanil concentration of 38 ng/ml and at a sevoflurane concentration of 0.7 ET%. The combinations of sevoflurane and alfentanil causing 25, 50, and 75% reduction in \dot{V}_i are given in figure 2 (*TOP Right*). The synergy is obvious from the concave form of the isoboles. One possibility for 25% reduction is 13.4 ng/ml alfentanil plus 0.12 ET% sevoflurane ($I(Q) \sim 1.9$ at a Q of ~ 0.7).

DISCUSSION

The main findings in this study are as follows: (1) Alfentanil, (up to a plasma concentration of 50 ng/ml), and sevoflurane (from 0 to 0.4 ET%), when administered separately, depress ventilation, HR, and the ventilatory and HR responses to acute hypoxia in a dose-dependent linear manner; (2) When combined, their depressant effect on ventilation and HR is synergistic, whereas their effect on the hypoxic responses is additive; (3) Relative to normoxic baseline parameters (\dot{V}_i , V_T , RR and HR), the responses to hypoxia, show greater sensitivity to the effects of alfentanil and sevoflurane (*i.e.*, depression occurs at lower drug concentrations), when drugs are administered separately and when combined (C_{25} values differ by 2–8 times); (4) The bispectral index is sensitive to sevoflurane but not alfentanil, even when these agents are combined (inert interaction).

The Pharmacodynamic Model

Although the relation between drug concentration and respiratory effect has been modeled previously using an inhibitory sigmoid E_{\max} model,⁴² we refrained from such an approach. In contrast, we chose the function $f(x) = a(1 - x^y)$, which allows for linear and non-linear concentration-effect relationships (see fig. 6). This was done for the following reasons:

1. Asymmetric sigmoidal relations between drug and effect may occur in complex systems, such as the ventilatory control system. For example, at high drug concentrations (higher than those studied by us), nonlinear threshold values may cause

Table 2. Model Parameter Estimates for Ventilation (\dot{V}_i), Tidal Volume (V_T), Respiratory frequency (RR), heart rate (HR), the hypoxic sensitivities ($\Delta E/\Delta S_{pO_2}$) and bispectral index (BIS)

	\dot{V}_i L/min	$\Delta\dot{V}_i/\Delta S_{pO_2}$ L min ⁻¹ % ⁻¹	HR min ⁻¹	$\Delta HR/\Delta S_{pO_2}$ min ⁻¹ % ⁻¹
Baseline	18.4 ± 1.5	1.04 ± 0.27	60.7 ± 2.4	1.10 ± 0.10
%CV	22	74	11	24
<i>C</i> ₂₅ Alfentanil ^a	37.7 ± 11	15.7 ± 1.4	155 ± 90	19.1 ± 3.0
<i>C</i> ₅₀ Alfentanil ^a	75.3 ± 23	31.3 ± 2.8	310 ± 180	38.2 ± 6.1
%CV	48	17	108	20
<i>C</i> ₂₅ Sevoflurane ^b	0.73 ± 0.44	0.14 ± 0.05	1.92 ± 1.35	0.25 ± 0.11
<i>C</i> ₅₀ Sevoflurane ^b	1.46 ± 0.88	0.27 ± 0.05	3.85 ± 2.60	0.50 ± 0.23
%CV	106	41	*	70
<i>I</i> _{max}	1.92 ± 0.28		2.47 ± 0.90	
<i>Q</i> _{max}	0.68 ± 0.11		0.69 ± 0.13	
SD of Residuals	2.99	0.21	3.67	0.33

	V_T ml	$\Delta V_T/\Delta S_{pO_2}$ ml % ⁻¹	RR min ⁻¹	$\Delta RR/\Delta S_{pO_2}$ min ⁻¹ % ⁻¹	BIS
Baseline	1150 ± 50	46 ± 11	16.1 ± 1.1	0.25 ± 0.07	96.3 ± 0.8
%CV	11	62	16	86	2.1
<i>C</i> ₂₅ Alfentanil ^a	37.0 ± 6.5	18.4 ± 2.6	81.5 ± 24.7	11.6 ± 0.4	*
<i>C</i> ₅₀ Alfentanil ^a	74.0 ± 13.0	36.8 ± 5.2	163.0 ± 49.3	23.2 ± 0.7	*
%CV	30	28	*	*	*
<i>C</i> ₂₅ Sevoflurane ^b	0.39 ± 0.18	0.15 ± 0.05	*	0.24 ± 0.02	0.45 ± 0.08
<i>C</i> ₅₀ Sevoflurane ^b	0.79 ± 0.36	0.29 ± 0.10	*	0.49 ± 0.04	0.90 ± 0.17
%CV	80	70	*	*	53
SD of Residuals	80	10.9	2.49	0.18	3.32

Values are population estimate ± SE and the percentage coefficient of variation (%CV), which is a measure of the interindividual variability, as derived from the NONMEM analysis;

Baseline is the control parameter value (*i.e.*, before drug administration);

a and *b*: *C*₂₅ and *C*₅₀ denote concentrations of alfentanil (in ng/ml) or sevoflurane (in end-tidal concentration or *ET*%) giving 25% and 50% reductions in *E*;

* parameter not included in the statistical model;

*I*_{max} and *Q*_{max} are interaction parameters: *I*_{max} values not significantly different from 1 are not included in the table (in these cases the alfentanil–sevoflurane interaction is additive) and *I*_{max} values greater than 1 indicate synergy;

SD (standard deviation) of the residuals is a measure of goodness of fit.

the respiratory oscillator to stop abruptly and may cause irregular or cyclic breathing and apnea.¹⁹⁰ In our model effect becomes zero at the concentration $C_{50} \cdot 2^{\frac{1}{\gamma}}$ (fig. 6).

2. Some respiratory parameters, such as hypoxic sensitivity ($\Delta\dot{V}_i/\Delta S_pO_2$), may become negative above certain drug concentrations. For example, we previously observed in one male subject that although hypoxia caused an increase in \dot{V}_i during alfentanil infusion (target, 40 ng/ml) in the awake state, it caused an immediate decrease in ventilation when the subject was asleep (*i.e.*, $[\dot{V}_i(\text{hypoxia}) - \dot{V}_i(\text{normoxia})]/[S_pO_2(\text{normoxia}) - S_pO_2(\text{hypoxia})] < 0$).¹⁷⁰ Our model predicts such behavior at concentrations $> C_{50} \cdot 2^{\frac{1}{\gamma}}$ (figure 6). In some of the subjects in this study negative responses were observed for $\Delta V_T/\Delta S_pO_2$, $\Delta RR/\Delta S_pO_2$ and $\Delta HR/\Delta S_pO_2$ at high alfentanil concentrations.
3. Within a limited dose range (such as the range studied by us) some respiratory dose-response relations appear linear or almost linear. For example, halothane and isoflurane, over the dose range from 0 to 0.2 MAC, cause a linear decrease of the hypoxic ventilatory response (at 0.2 MAC $\Delta\dot{V}_i/\Delta S_pO_2$ is approximately 20% of control).^{47,201} In our model, a linear function is obtained when $\gamma = 1$ (fig. 6). Interestingly, for all parameters our model, fits yielded values of γ not significantly different from 1. This indicates that, at least within the dose range we tested, the data are well-described by a linear relation between drug (sevoflurane or alfentanil) and effect. This is also true for combinations of both agents, but only at fixed concentration ratios (Q). Incorporation of higher doses might have yielded values of γ significantly different from 1. This may be especially true for the BIS-sevoflurane relation.¹³⁷

Response Surface Modeling The response surface modeling method, as recently reported by Minto *et al.*,¹²⁹ is based on two ideas. First, there is the notion that the combination of two drugs should be regarded as a new drug with its own properties,¹⁸³ with the concentration-effect relation $E = f(C_A/C_{50,A} + C_S/C_{50,S}; \psi)$ where the parameter vector ψ (in our model $I(Q)$) controls the properties of the interaction and specifically how it deviates from pure additivity. Second, ψ is assumed to depend only on the ratio of the concentrations of the two administered drugs.²⁸ These two ideas are crucial because they allow for a greatly reduced number of parameters necessary to describe a surface and thus make them estimable from a study of reasonable size.¹²⁹ A proper choice of ψ and f may further reduce the number of parameters while describing the concentration-effect relation in the range measured. We made two modifications to the model specified by Minto *et al.*. First, interaction was taken into account by the function $I(Q)$ for which we chose a spline with two, interpretable parameters (see Appendix 1 for details). Second, for f we chose equation 1.

Although visual inspection of the residuals did not show remaining structure, other possibilities of modeling the concentration-effect relation and the nature of the inter-

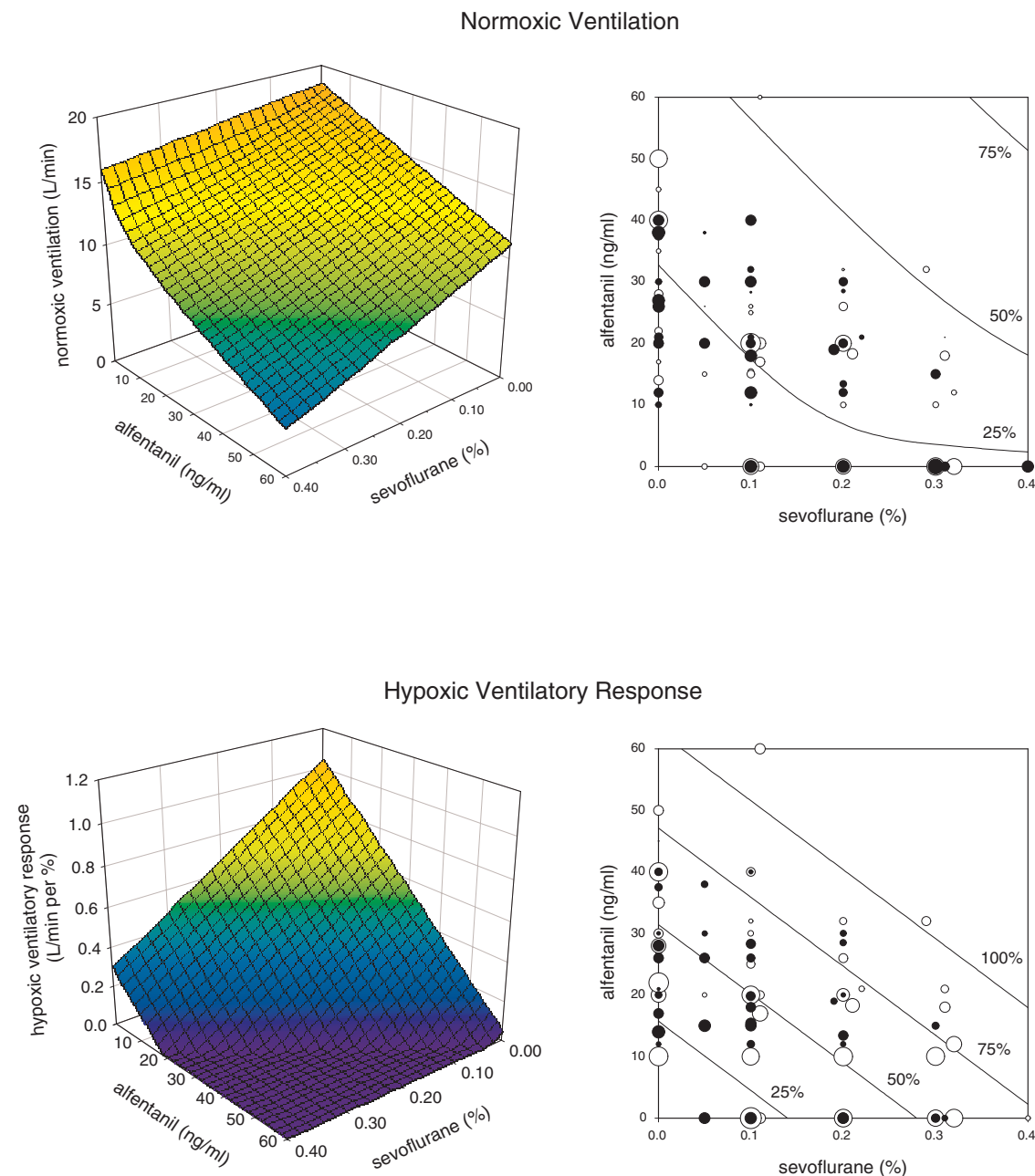


Figure 2. *TOP.* Left: Response surface modeling of the influence of plasma alfentanil concentration and end-tidal sevoflurane concentration on normoxic ventilation (\dot{V}_i) at a fixed $P_{ET}CO_2$. Right: Individual data points and isoboles of 25, 50, and 75% depression of \dot{V}_i . Open circles denote data points above the surface, closed circles denote data points below the surface (control data points not shown). The area of the circles is proportional to the distance from that data point to the surface area. A 25% reduction of \dot{V}_i occurred at: an alfentanil concentration of ~ 38 ng/ml when no sevoflurane was present, at a sevoflurane concentration of $\sim 0.73\%$ when no alfentanil was present, and at $\sim 0.1\%$ sevoflurane when 15 ng/ml alfentanil was present. This indicates synergistic interaction ($I(Q) \sim 1.9$ at Q of ~ 0.7 ; $P < 0.01$). *BOTTOM.* Left: Response-surface modeling of the influence of alfentanil and sevoflurane on the ventilatory response to acute hypoxia at a fixed $P_{ET}CO_2$ (i.e., the hypoxic drive). Right: Individual data points and isoboles of 25, 50, 75 and 100% reduction of hypoxic drive. A 25% reduction of hypoxic drive occurred at an alfentanil concentration of ~ 16 ng/ml when no sevoflurane was present, at a sevoflurane concentration of $\sim 0.14\%$ when no alfentanil was present, and at $\sim 0.05\%$ sevoflurane when 10 ng/ml alfentanil was present. This indicates additive interaction.

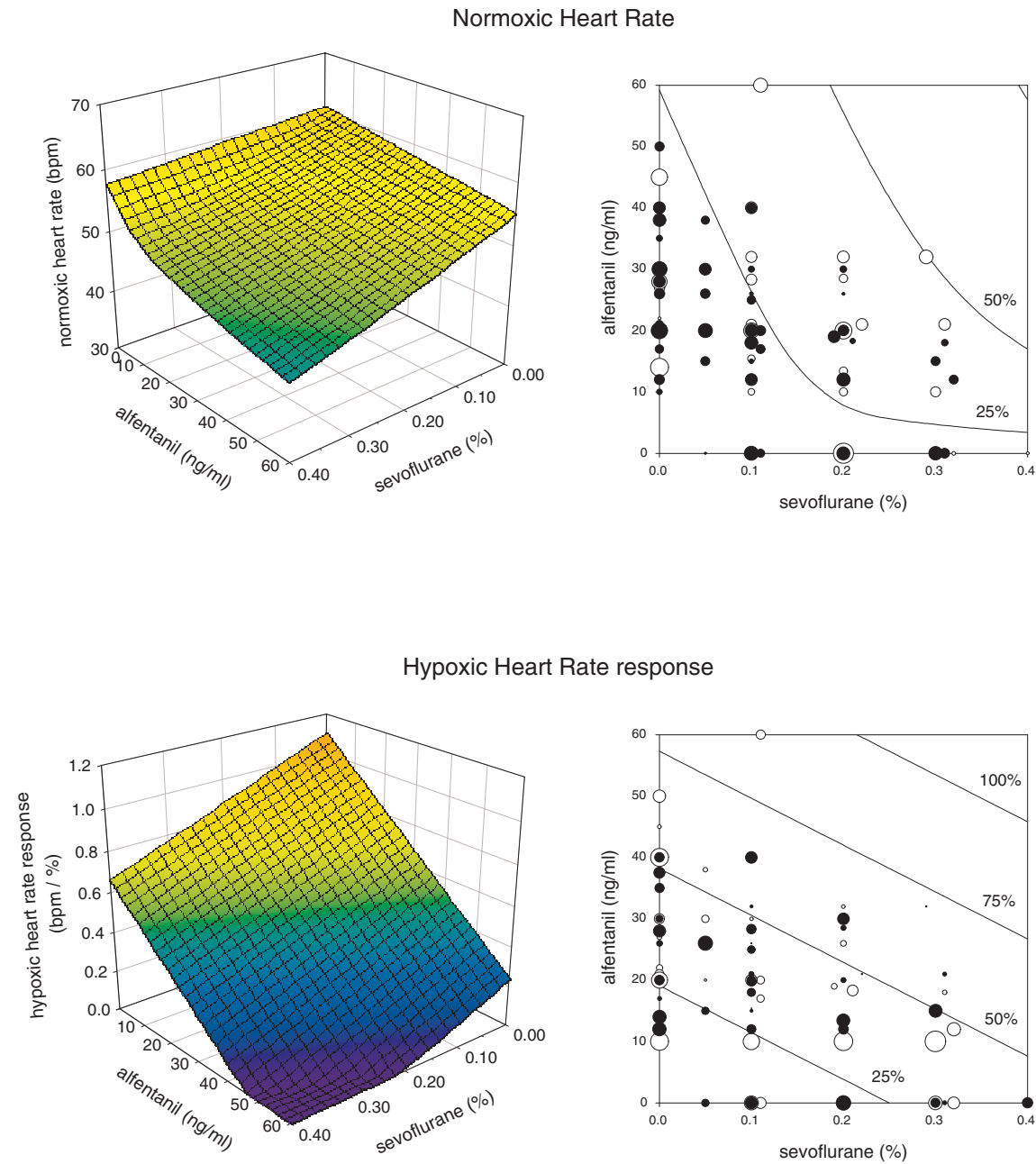


Figure 3. *TOP.* Left: Response surface modeling of the influence of plasma alfentanil concentration and end-tidal sevoflurane concentration on normoxic heart rate (HR) at a fixed $P_{ET}CO_2$. Right: Individual data points and isoboles of 10, 20, and 30% reduction of HR. Open circles denote data points above the surface, closed circles denote data points below the surface (control data points not shown). The area of the circles is proportional to the distance from that data point to the surface area. *BOTTOM.* Left: Response-surface modeling of the influence of alfentanil and sevoflurane on the HR response to acute hypoxia at a fixed $P_{ET}CO_2$. Right: Individual data points and isoboles of 25, 50, 75, and 100% reduction of the HR response to acute hypoxia at a fixed $P_{ET}CO_2$.

action could be explored, for example, by using the function:^{157,†}

$$(8) \quad f(x) = \frac{\alpha}{(1 + \delta \cdot x^\gamma)^{1/\delta}}$$

Now a spectrum of linear, sigmoidal, and asymmetric sigmoidal concentration-effect relations is possible with varying values of γ and δ . However, because in our analysis γ never significantly differed from 1, inclusion of additional nonlinear parameters, such as in equation 8, does not seem necessary. Finally, when synergistic interactions were detected (*i.e.*, for \dot{V}_i and HR), the standard deviations of the estimated parameters did not warrant a more complex form of $I(Q)$.

\dot{V}_i and HR versus $\Delta\dot{V}_i/\Delta S_pO_2$ and $\Delta HR/\Delta S_pO_2$

We observed synergistic alfentanil–sevoflurane interactions on \dot{V}_i and HR. This indicates that the effect of the drug combination was greater than expected from the dose-response curves of sevoflurane alone and alfentanil alone. Our analysis shows that for both variables, maximum synergy occurred at Q values of approximately 0.7. This indicates that, taking into account their respective C_{50} values, concentrations that give maximal synergy on reduction of \dot{V}_i are at fractions (or multiples) of 26.7 ng/ml for alfentanil and 0.24 ET% for sevoflurane (maximum synergy causing 25% reduction of ef-

[†]Note that when δ is fixed to -1, equation. (8) reduces to eqn. 1.

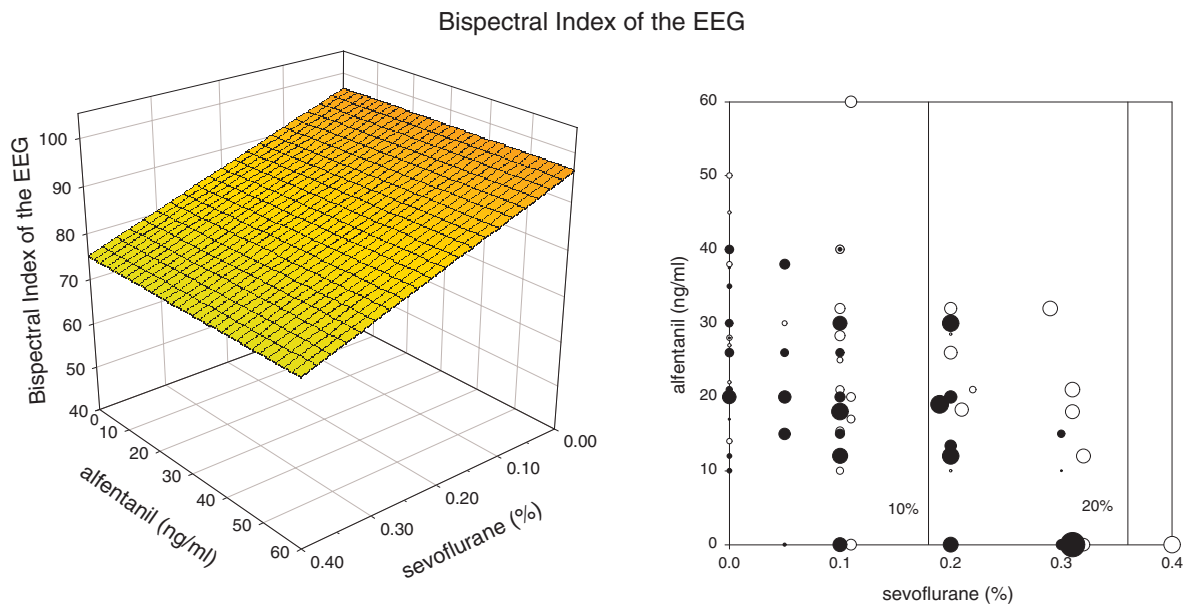


Figure 4. *LEFT.* Response surface modeling of the influence of plasma alfentanil concentration and end-tidal sevoflurane concentration on the Bispectral Index (BIS). *RIGHT.* Individual data points and isoboles of 10, and 20% reduction of the BIS. Open circles denote data points above the surface, closed circles denote data points below the surface (control data points not shown). The area of the circles is proportional to the distance from that data point to the surface area. A 25% reduction of the BIS occurred at a sevoflurane concentration of ~0.43% (table 2), but no decrease was seen with increasing doses of alfentanil, even in the presence of sevoflurane.

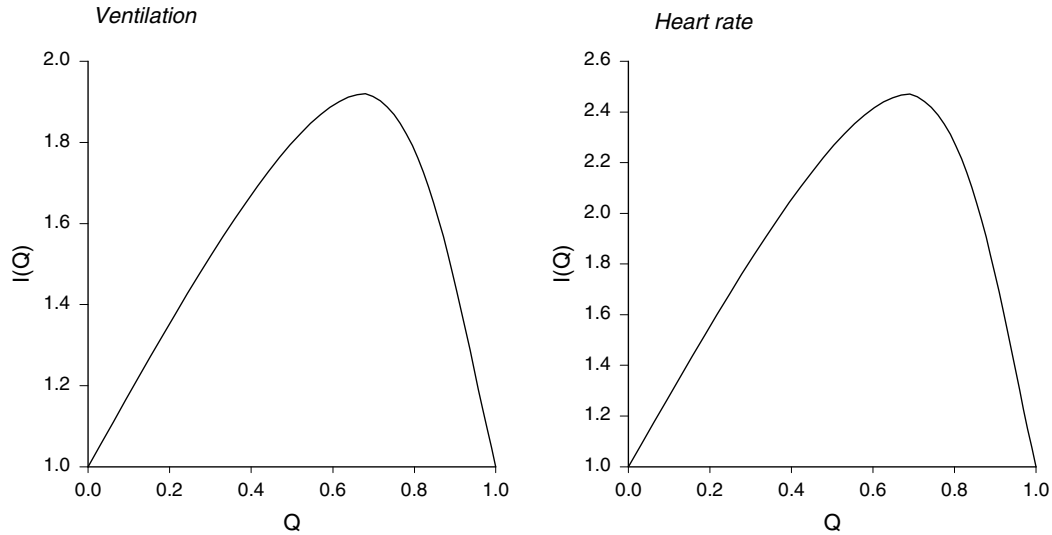


Figure 5. The relations between Q (i.e., the alfentanil-sevoflurane concentration ratio) and $I(Q)$ (i.e., the interaction) for ventilation (left) and heart rate (right). For both effects, synergistic interactions were observed over the range of concentration ratios from 0 to 1, with maximum synergistic interactions (I_{\max} in table 2) occurring at Q values of about 0.7 (Q_{\max} in table 2).

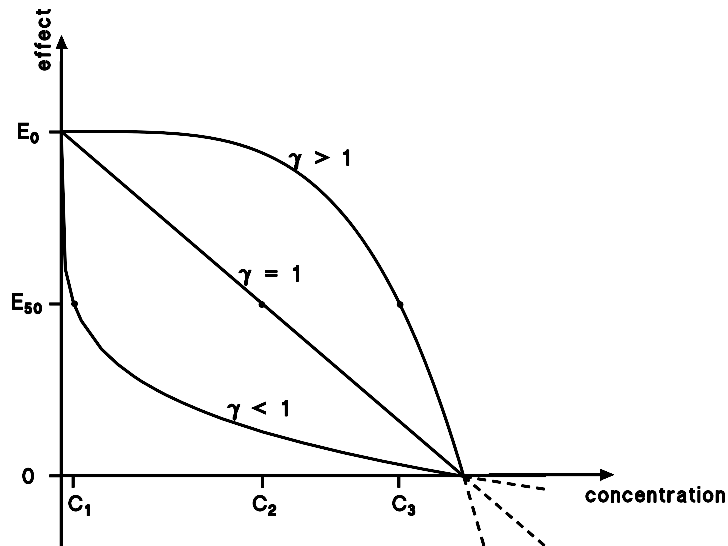


Figure 6. Three examples of the concentration-effect relation predicted by the pharmacodynamic model $f(x) = E_0(1 - x^\gamma)$ where $x^\gamma = U^\gamma/2$ and $U = C/C_{50}$, obtained at three fixed values of γ (0.2, 1 and 4). when $\gamma = 1$, the model becomes linear. At all three γ values, negative effects are possible at drug concentrations $x > C_{50} \cdot 2^{\frac{1}{\gamma}}$. Note that the values for C_{50} and γ were chosen in such a way that the zero crossing for all three examples occurred at the same value of C . As a consequence the values of C causing 50% of the effect (i.e., C_{50}) occur at different values of C (C_1 through C_3 are the C_{50} values for the three different curves). Different values for C_{50} and γ would result in the zero crossing at different values of C . E_0 = baseline effect, E_{50} = 50% depression of effect.

fect occurs at 13.4 ng/ml alfentanil + 0.12 ET% sevoflurane, maximum synergy causing 50% reduction occurs at 26.7 ng/ml alfentanil + 0.24 ET% sevoflurane, and maximum synergy causing 100% reduction occurs at 53.4 ng/ml alfentanil + 0.48 ET% sevoflurane).[‡] For HR these concentrations are fractions of 86.6 ng/ml for alfentanil and 0.48 ET% for sevoflurane (= C_{50} values). Because we explored only a relatively small part of the response surface for \dot{V}_i and HR, C_{50} and C_{100} values are extrapolations. As indicated above, nonlinearities of the ventilatory controller may cause apnea at concentrations higher than those explored in our protocol but lower than those estimated from our analysis. Moreover, we may have underestimated the magnitude of synergy in our study. Therefore, our results apply to the portion of the response surface analyzed and extrapolation should be performed with caution (especially for those surfaces that are explored only marginally, *e.g.*, HR). The mechanism of the observed synergistic interactions remain elusive. Further studies (*e.g.*, identifying shared central effect-sites for opioid- and anesthetic-induced respiratory depression) are needed to understand the synergistic effects of alfentanil and sevoflurane on ventilation and heart rate.

We observed additive alfentanil-sevoflurane interactions on $\Delta\dot{V}_i/\Delta S_pO_2$ and $\Delta HR/\Delta S_pO_2$. This indicates that the effect of the sevoflurane-alfentanil combination is expected from the concentration-response curve of the individual agents. The absence of synergy may be related to the different pathways through which sevoflurane and alfentanil depress the hypoxic response (inhalational anesthetics depress hypoxic response at sites within the peripheral chemoreflex loop,^{47,201} and opioids depress the hypoxic response through effects at the brainstem). Another possibility is that because of the great sensitivity of the hypoxic response to both agents and consequently the early loss of the hypoxic response (*i.e.*, $\Delta\dot{V}_i/\Delta S_pO_2 = 0$ occurring at 60 ng/ml alfentanil and 0.5 ET% sevoflurane), we are unable to unearth any synergy from the data.

The observation that the hypoxic drive, relative to resting \dot{V}_i , is more sensitive to the effects of an opioid and an inhalational anesthetic, suggests that the hypoxic test is the more sensitive tool to assess the effects of anesthetics and opioids on ventilatory control.

One of the many determinants of HR at rest and during hypoxia is stimulation and depression of lung receptors by increases/ and decreases in \dot{V}_i (*i.e.*, HR effects are secondary to \dot{V}_i -effects).[§] This may explain the agreement in the nature of alfentanil-sevoflurane interactions for \dot{V}_i and HR and $\Delta\dot{V}_i/\Delta S_pO_2$ and $\Delta HR/\Delta S_pO_2$.

Bispectral Index and Respiration

In contrast to sevoflurane, alfentanil, in the absence and presence of sevoflurane, had no effect on BIS. This indicates that the algorithm for calculating the BIS is not sensitive to the changes in arousal level from opioids and the combination of opioids and anesthetics (at least within the dose-ranges we evaluated). Because we did not obtain other measures of sedation during the study (such as the subjective observers' assess-

[‡]These values are obtained by simultaneously solving equations 6 and 7, with $E/E_0 = 0.75$ for 25% reduction, $E/E_0 = 0.5$ for 50% reduction and $E/E_0 = 0$ for 100% reduction.

[§]see Chapter 4

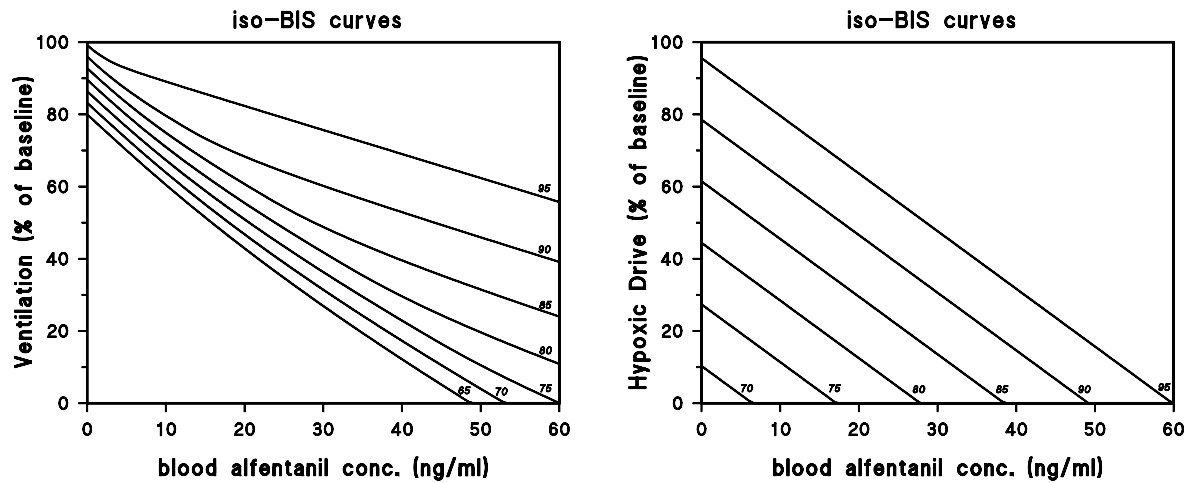


Figure 7. The influence of alfentanil on ventilation (*left.*) Values are % of control) and hypoxic drive (*right.*) Values are % of control) at constant bispectral index (BIS) values (so called iso-BIS curves). The BIS values are in italic and range from 95 to 65. The BIS values were fixed by inhalation of constant concentration of sevoflurane. Note that the hypoxic drive is more vulnerable than \dot{V}_i for the effects of alfentanil at all tested BIS values.

ment of alertness and sedation score) we remained uninformed on the level of arousal during the alfentanil-sevoflurane studies. However, because clinicians and researchers use BIS monitoring, we constructed iso-BIS curves (equivalent to iso-effect curves, fig. 7). We now are informed about the steady-state relation between alfentanil and \dot{V}_i and $\Delta\dot{V}_i/\Delta S_{pO_2}$ at varying BIS levels. Further studies are needed to examine whether these steady-state relations are independent of sevoflurane and also apply to other agents that affect the BIS (such as propofol, isoflurane, dexmedetomidine, midazolam), or that separate BIS-opioid- \dot{V}_i -relations exist for each anesthetic/hypnotic.

Study Limitations

Some methodologic issues deserve further comment. We used an isohypercapnic design (P_{ETCO_2} fixed to ~ 48 mmHg). This enables the comparison of the effect of various drug concentrations on \dot{V}_i without the confounding influence of variations in P_{ETCO_2} . However, because every drug effect on \dot{V}_i is conditional on a certain P_{ETCO_2} value, other results might be seen at different carbon dioxide concentrations. Furthermore, because we performed a steady-state study (drug concentrations, P_{ETO_2} , and P_{ETCO_2} were clamped during hypoxic testing), the time course of drug-induced variations in blood gases (such as those that may occur during bolus opioid infusions) and their translation into \dot{V}_i and oxygen delivery were not modeled. Further studies are needed to examine the blood gas and \dot{V}_i dynamics caused by the administration of opioids and anesthetics occurring in the clinical setting.

APPENDIX

For the function $I(Q)$ we use a spline with a piece $g_1(x)$ between knots at $x = 0$ and $x = Q_{max}$ and a piece $g_2(x)$ between knots $x = Q_{max}$ and $x = 1$, constrained by the following eight conditions:

$$\begin{aligned}
 (9) \quad & g_1(0) = g_2(1) = 1 \\
 (10) \quad & g_1(Q_{max}) = g_2(Q_{max}) = I_{max} \\
 (11) \quad & dg_1(x)/dx \Big|_{x=Q_{max}} = dg_2(x)/dx \Big|_{x=Q_{max}} = 0 \\
 (12) \quad & d^2g_1(x)/dx^2 \Big|_{x=0} = d^2g_2(x)/dx^2 \Big|_{x=1} = 0
 \end{aligned}$$

The first four constraints (eqns. 9 and 10) deal with the values of $I(Q)$ at the three knots, constraints five and six (eqn. 11) make the second knot a maximum (or minimum) and constraints seven and eight (eqn. 12) are natural boundary conditions at the first and last knot and assure the absence of values outside $(1, I_{max})$. Because we specify eight conditions, we can utilize a cubic spline so that each piece is given by a third-order polynomial:

$$(13) \quad g(x) = a_0 + a_1x + a_2x^2 + a_3x^3$$

The parameters a_i are such that the above constraints are satisfied. Note that they need not be interpreted, but are merely functions of I_{max} and Q_{max} . For $g_1(x)$ we have:

$$\begin{aligned}
 a_0 &= 1 \\
 a_1 &= -3(1 - I_{max})/(2Q_{max}) \\
 a_2 &= 0 \\
 (14) \quad a_3 &= (1 - I_{max})/(2Q_{max})/Q_{max}^2
 \end{aligned}$$

For $g_2(1 - x)$, the parameters a_i are obtained by substituting $(1 - Q_{max})$ for Q_{max} .

7 Response surface modeling of remifentanil–propofol interaction on cardiorespiratory control and bispectral index

THE COMBINED administration of opioids and anesthetics for induction and maintenance of anesthesia is common practice. The anesthetic is given to lose consciousness, prevent awareness and reduce movement responses, the opioid is given to suppress somatic, stress and adrenergic responses to surgical stimulation. An important advantage of combining an opioid and an anesthetic is the synergistic increase in these desired effects, with consequently the need for less drugs to attain the goal of adequate anesthesia relative to the amount of drug needed when only a single agent (*i.e.*, an anesthetic) is given.¹¹⁰ Since this is not only true for patients that are intubated and ventilated but also for patients that maintain their own breathing, for example during ‘monitored anesthesia care’, it is of interest to address the issue of the effect of drug combinations on respiration. While it is known that anesthesia induces many ‘side effects’ it is acknowledged that respiratory depression is potentially life-threatening.¹²³ We therefore studied the effect of the opioid remifentanil and intravenous anesthetic propofol on the cardiorespiratory control. This combination of drugs is frequently used in patients under monitored anesthesia care for minor (without additional regional anesthesia) and major (with additional regional anesthesia) surgery. Knowledge on the quantitative and qualitative (additive *versus* synergistic) nature of their interaction is clinically important and may lead to specific dosing regimens aimed at the titration of sedation/analgesia *versus* respiratory effect.

To study the remifentanil-propofol interaction, we made use of the technique of response surface modeling.^{82,89,129,191} This technique allows the observation of the concentration-effect relation among infinite combinations of remifentanil and propofol over the whole surface area in three dimensional space. In *Chapter 6* we made successful use of this technique to quantify the interactive effects of sevoflurane and alfentanil on cardiorespiratory control.

METHODS

Subjects

Twenty-two healthy male volunteers (aged 19–25 yr) participated in the protocol after approval was obtained from the local Human Ethics Committee (Commissie Medische Ethiek, Leiden University Medical Center, 2300 RC Leiden, The Netherlands). Oral and written consent was obtained from all volunteers.

Table 1. Results of the bootstrap based model selection

	MODEL																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	non-linear*	interaction**					
Resting \dot{V}_i	0	0	0	1	1	68	531	81	74	46	3	145	12	5	33	929	998					
\dot{V}_{55}	0	28	32	1	22	0	431	37	0	268	0	128	1	0	47	1000	912					
Resting $P_{ET}CO_2$	53	29	56	11	41	130	357	150	35	68	10	31	13	7	9	717	810					
S	22	81	16	24	16	3	357	18	4	261	1	106	2	3	86	974	841					
Heart rate	156	240	133	121	20	69	210	7	8	0	6	10	0	1	0	769	311					
MAP	664	3	13	6	11	140	81	9	18	2	3	23	4	11	1	193	292					

Analysis was performed on 1000 data sets based on the original 22 studies created by the bootstrap method;

The values are the number of times that specific model was chosen using Akaike's Information-theoretic Criterion (AIC);

Bold numbers are the most frequent chosen ones. The corresponding model was used in the analysis of the original data set.

* and ** Total number of times that a non-linear (models 2-5 + 7-10 + 12-15) or a non-additive interaction model (models 6-15) was chosen;

\dot{V}_{55} is ventilation at a fixed $P_{ET}CO_2$ of 55 mmHg; S is the slope of the hypercapnic ventilatory response.

MAP is mean arterial pressure.

Apparatus

After arrival at the laboratory, an intravenous catheter was inserted in the left antecubital vein (for drug infusion) and an arterial line was placed in the right radial artery (for blood sampling). Subsequently, electrodes for EEG monitoring (BisSensor, Aspect Medical Systems, Newton, MA) were placed on the head as specified by the manufacturer and the subjects rested for 20 to 30 min. Next a face mask was applied over the mouth and nose and data collection started.

See METHODS section *Apparatus* of *Chapter 2* for a description of the procedure and apparatus. The EEG was recorded using an Aspect A-2000 EEG monitor (software version 3.3). The monitor computed the bispectral index (BIS) over 2-s epochs. We averaged the BIS values over 1 min-intervals.

Study Design

Resting ventilation and $P_{ET}CO_2$ (i.e., without any inspired CO_2), blood pressure, heart rate, BIS and the ventilatory response to hypercapnia were measured before and during infusion of remifentanil, propofol and the combined infusion of these agents. Initially control (i.e. without the administration of any agent) values were obtained. Next the infusion of remifentanil was started and cardiorespiratory and BIS parameter values were obtained at steady state blood target concentrations. After this set of studies, the infusion was terminated and the subject rested for 1 hour. Next the infusion of propofol was started and cardiorespiratory and BIS parameter values were obtained at steady state blood target concentrations. Subsequently parameter values were obtained during the combined administration of remifentanil and propofol. In some subjects two to three studies were performed at different propofol-remifentanil combinations. The subjects were randomly assigned to a fixed scheme of target concentrations of remifentanil and propofol. The scheme was designed to ensure that, over the applied dose ranges, evenly spread data points were obtained.

The Ventilatory Response to Hypercapnia

The ventilatory response to CO_2 was obtained by using the ‘dynamic end-tidal forcing’ technique.³⁸⁻⁴⁰ After assessment of resting variables, 3 to 8 elevations in $P_{ET}CO_2$ were applied to obtain data points for the steady-state ventilatory response. The elevations varied from 3 to 19 mmHg. The elevated $P_{ET}CO_2$ readings lasted at least 8 min. When on-line analysis revealed that a ventilatory steady-state had not been reached, the duration of hypercapnia was extended. The order of elevations was arbitrarily chosen. All hypercapnic studies were performed at a background of moderate hyperoxia ($P_{ET}O_2$ 120 mmHg).

The elevated $P_{ET}CO_2$ and the corresponding \dot{V}_i breath-to-breath data were averaged over 10-breaths. Data points were obtained at the end of the $P_{ET}CO_2$ elevation. This procedure yielded 3 to 8 steady-state data points. We expressed ventilation as a linear function of $P_{ET}CO_2$: $\dot{V}_i = S (P_{ET}CO_2 - B_k)$, where S is the ventilatory CO_2 sensitivity and B_k the extrapolated $P_{ET}CO_2$ at zero \dot{V}_i . Parameters S and B_k were determined by linear regression of \dot{V}_i on $P_{ET}CO_2$.

Remifentanil and Propofol Administration, Blood Sampling and Assays.

Propofol and remifentanil were administered using target controlled infusion (TCI) systems. For propofol we used a Psion palm-top computer (London, England) programmed with a three compartment propofol pharmacokinetic (PK) data set to control a Becton Dickinson infusion pump (St. Etienne, France).^{70,79} For remifentanil we used a custom build infusion pump which

Table 2. Population Pharmacodynamic Estimates

	\dot{V}_i L/min	\dot{V}_{55} L/min	$P_{ET}CO_2$ mmHg	S L min ⁻¹ mmHg ⁻¹	MAP mmHg	HR min ⁻¹	BIS
Baseline value	9.4 ± 0.3	31.4 ± 1.5	41.2 ± 0.1	1.87 ± 0.01	93.0 ± 1.6	64.1 ± 1.8	96.4 ± 0.4
95% c.i.	8.8 - 10.0	28.4 - 34.4	41.0 - 41.4	1.84 - 1.89	89.8 - 96.2	60.4 - 67.7	95.6 - 97.2
λ remifentanyl (%)	27.7 ± 3.5	57.7 ± 3.5	15.4 ± 1.2	20.0 ± 5.4	3.7 ± 1.1	10.6 ± 2.7	-
95% c.i.	20.7 - 34.7	51.0 - 65.0	13.0 - 17.8	9.2 - 30.8	1.5 - 5.9	5.2 - 16.0	-
λ propofol (%)	12.6 ± 3.3	44.3 ± 3.9	4.2 ± 0.9	51.0 ± 4.5	9.9 ± 1.8	11.9 ± 3.1	18.9 ± 1.4
95% c.i.	6.0 - 19.2	37.0 - 52.0	2.4 - 6.0	42.0 - 60.0	6.3 - 13.5	5.7 - 18.1	16.1 - 21.7
I_{max}	1.9 ± 0.2	1.2 ± 0.1	1.3 ± 0.2	1.3 ± 0.1	1	1	-
95% c.i.	1.5 - 2.3	1.04 - 1.38	0.9 - 1.7	1.1 - 1.5			
Q_{max}	0.5	0.5	0.5	0.5	0.5	0.5	-
γ	0.5 ± 0.1	0.4 ± 0.05	0.7 ± 0.1	0.4 ± 0.1	1	0.3 ± 0.1	1
95% c.i.	0.3 - 0.7	0.27 - 0.47	0.5 - 0.9	0.2 - 0.6		0.1 - 0.5	
$C_{50,R}$ (ng/ml)	3.3	0.7	5.4	8.6	13.5	142.0	-
$C_{50,P}$ (μg/ml)	15.8	1.4	34.4	1.0	5.1	98.1	2.7

Values are population estimate ± SE and 95% confidence interval (c.i.) as derived from the NONMEM analysis;

λ is the % decrease at 1 ng/ml remifentanyl or 1 μg/ml propofol;

$C_{50,R}$'s and $C_{50,P}$'s are extrapolated values;

\dot{V}_i is resting ventilation; \dot{V}_{55} is ventilation at a fixed $P_{ET}CO_2$ of 55 mmHg;

S is the slope of the hypercapnic ventilatory response; MAP is mean arterial pressure;

I_{max} and Q_{max} are interaction parameters (see text): I_{max} values > 1 indicate synergy, = 1 additivity.

was programmed with a remifentanyl pharmacokinetic data set (Remifusor, University of Glasgow, Glasgow).¹²⁸ These systems allow a specified target plasma concentration of remifentanyl and propofol to be rapidly achieved and maintained. Hypercapnic studies were performed ~10 min after blood remifentanyl and propofol concentrations had reached their target levels. Since this equals >5-10 times the remifentanyl and propofol blood–effect-site equilibration half-lives, we assumed that brain and blood remifentanyl and propofol concentrations were in equilibrium.

Before and after changes in target drug concentrations, arterial blood samples for determination of remifentanyl and propofol concentrations were collected. Blood for propofol determination was collected in syringes containing potassium oxalate. Propofol concentrations were determined by reverse-phase high performance liquid chromatography.¹³² Samples for the determination of blood remifentanyl concentrations were collected into tubes containing sodium heparin and immediately transferred to tubes containing 50% citric acid (to inactivate esterases) before freezing at –20°C. The assay method is based on tandem mass spectrometry detection.¹⁰

Response Surface Modeling

Analysis was performed on the following parameters: resting inspired minute ventilation (\dot{V}_i) and $P_{ET}CO_2$ (i.e., without any inspired CO_2), slope of the hypercapnic ventilatory response (S), ventilation at a fixed $P_{ET}CO_2$ of 55 mmHg (\dot{V}_{55} , calculated from S and B_K), mean arterial pressure (MAP), heart rate (HR) and BIS. The basis of the pharmacodynamic (PD) model is similar to the model described in *Chapter 6*. The single-drug concentration (C) – effect (E) relationship is given by

$$(1) \quad E(C) = E_0 \cdot \left\{ 1 - \left(\frac{C}{C_{50}} \right)^y \cdot \frac{1}{2} \right\}$$

where E_0 is the baseline drug effect, C_{50} the value of C which gives 50% depression, and y a nonlinearity parameter; notice that the model is linear when $y=1$. A straightforward extension for two concomitantly administered drugs (C_r = remifentanyl concentration, C_p = propofol concentration) is obtained by respecting Loewe additivity:¹³

$$(2) \quad E(C_r, C_p) = E_0 \cdot \left\{ 1 - \left[\frac{C_r}{C_{50,r}} + \frac{C_p}{C_{50,p}} \right]^y \cdot \frac{1}{2} \right\}$$

Note that isoboles in the $C_r - C_p$ plane are straight lines, irrespective of the value of y . Deviations from additivity can be modeled as:

$$(3) \quad E(C_r, C_p) = E_0 \cdot \left\{ 1 - \left[\frac{C_r}{C_{50,r}} + \frac{C_p}{C_{50,p}} \right]^{y(Q)} \cdot \frac{1}{2} \cdot I(Q) \right\}$$

with $I(Q)$ a smooth function (spline) with a parameter denoting maximum interaction I_{\max} at $I(Q_{\max})$ and $Q = U_r/(U_r + U_p)$, $U_r = C_r/C_{50,r}$, $U_p = C_p/C_{50,p}$. To limit the number of parameters $y(Q)$ was either a constant or a linear function going from y_r at $Q = 1$ to y_p at $Q = 0$. Since the concentration ranges used in the study for most parameters lie below the C_{50} 's, these parameters will be poorly estimated leading to wide asymmetric confidence intervals. A remedy would be to use C_{10} 's or C_{25} 's but one doesn't know the optimal parameters beforehand. In fact, it is better to use parameters that are centered according to the study design:

$$(4) \quad E(C_r, C_p) = E_0 \cdot \left\{ 1 - \left[\frac{C_r}{C_{h,r}} \cdot \lambda_r^{1/y(Q)} + \frac{C_p}{C_{h,p}} \cdot \lambda_p^{1/y(Q)} \right]^{y(Q)} \cdot I(Q) \right\}$$

where $C_{h,r}$ and $C_{h,p}$ the values of C_r and C_p midway in the measured concentrations range, and Q redefined to be $Q = U_r / (U_r + U_p)$, $U_r = C_r / C_{h,r}$, $U_p = C_p / C_{h,p}$; λ_r and λ_p denote the degree of depression from E_0 when $C_r = C_{h,r}$ and $C_p = 0$ and vice versa, respectively. For parameter PCO_2 , which increases from E_0 , the model used was the same as eq. (4), except the minus sign was replaced by a plus sign.

Parameter Estimation and Model Selection

The above model has the following parameters to be estimated: E_0 , λ_r , λ_p , I_{\max} , Q_{\max} , γ_r and γ_p . The following situations are of special interest:

- $I_{\max} = 1$, $Q_{\max} = 0.5$ denoting additivity,
- $I_{\max} \neq 1$, $Q_{\max} = 0.5$ denoting symmetric interaction,
- $I_{\max} \neq 1$, $Q_{\max} \neq 0.5$ denoting asymmetric interaction.

Notice that when $Q_{\max} = 0.5$ we could use Minto's parabolic function of Q instead of the spline $I(Q)$.¹²⁹ Furthermore, when two drugs are pharmacodynamically equivalent apart from a difference in potency, we would expect a symmetric interaction (since Q is based on normalized concentrations). For each of the above three cases, there are five situations that describe (non)linearity:

- $\gamma_r = \gamma_p = 1$ denoting linearity,
- $\gamma_r = \gamma_p \neq 1$ denoting nonlinearity described by one parameter,
- $\gamma_r \neq 1$ and $\gamma_p = 1$ denoting nonlinearity for drug R and linearity for P ,
- $\gamma_r = 1$ and $\gamma_p \neq 1$ denoting linearity for drug R and nonlinearity for P ,
- $\gamma_r \neq 1$ and $\gamma_p \neq 1$ denoting nonlinearity described by two parameters.

This results in a total of fifteen models to be investigated (see fig. 1). NONMEM was used to estimate the parameter values.¹³⁵ Since the models are non-nested, the likelihood ratio criterion is not applicable so Akaike's Information-theoretic Criterion was used instead:¹³⁵ $AIC = -2LL + 2P$, where $-2LL$ is the minimum value of the objective function calculated by NONMEM and P denotes the number of parameters. The model with the lowest AIC is considered 'best'. The population analysis was done under the assumption of lognormally distributed model parameters and constant relative (except for PCO_2 where it was assumed to be additive) normally distributed intra-individual error.

Model Stability Assessment using the Bootstrap

When, according to AIC criterion, a model is chosen for a certain effect parameter, that choice is not associated with a measure of confidence in that model. One would like to be more certain that the choice is not an artifact of particular individuals in the current data set, and that when a new data set would be obtained, the same model would be chosen. A way to generate surrogate data sets is given by the method of the bootstrap.⁶⁹ Basically, a bootstrap data set is formed by selecting, with replacement, the data from individuals until a set is obtained with the same total number of individuals. This data set is then subject to the same fitting procedure, and by repeating the process N times, N parameter estimates are obtained with N selections of one

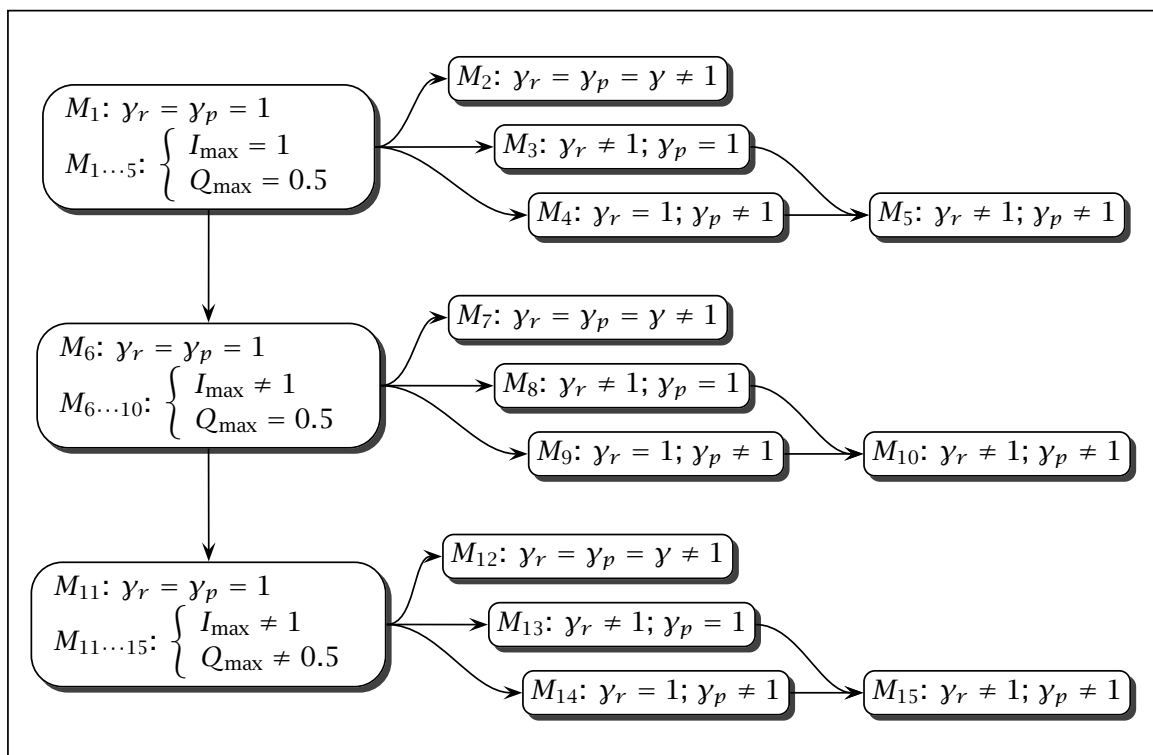


Figure 1. Schematic representation of the 15 different pharmacodynamic model (M) possibilities. Models 1 to 5: additive interaction between propofol and remifentanil; models 5 to 10: non-additive interactions at a value of Q_{\max} equal to 0.5; models 11 to 15: non-additive interactions at a value of Q_{\max} not equal to 0.5. Models 2, 7 and 12: linear relationships between propofol and remifentanil concentrations and effect; models 3, 8 and 14: a linear relationship between propofol concentration and effect, a non-linear relationship between remifentanil concentration and effect; models 4, 9 and 14: a non-linear relationship between propofol concentration and effect, a linear relationship between remifentanil concentration and effect. Models 5, 10 and 15: Non-linear relationships between propofol and remifentanil concentrations and effect.

of the fifteen models. From the parameter estimates confidence intervals and histograms can be constructed. The impact of constraining certain parameters to fixed values, and therefore identifiability, can then be studied visually. The number of times a model is selected is a measure of our confidence in the model.

The bootstrap procedure was implemented in a C++ program that generates bootstrap data sets, NONMEM control files with appropriately fixed parameters, runs NONMEM and reads back the estimated parameter values and the minimum value of the objective function. When NONMEM returned an error status regarding parameter boundary problems (despite carefully chosen initial conditions and boundaries) or rounding errors the model that was fitted was deemed to be not supported by the data. This, in principle, gives a bias towards the simpler models. Furthermore, to have a feasible procedure with respect to computer time, we opted not to investigate all possibilities for the statistical model. Initially, intra-individual variability was assumed to be present only on parameters E_0 , λ_r , and λ_p . When the number of times the corresponding variance was estimated to be negligible exceeded $N/2$, this variability term was removed and the bootstrap redone. Confidence intervals were obtained in the traditional way

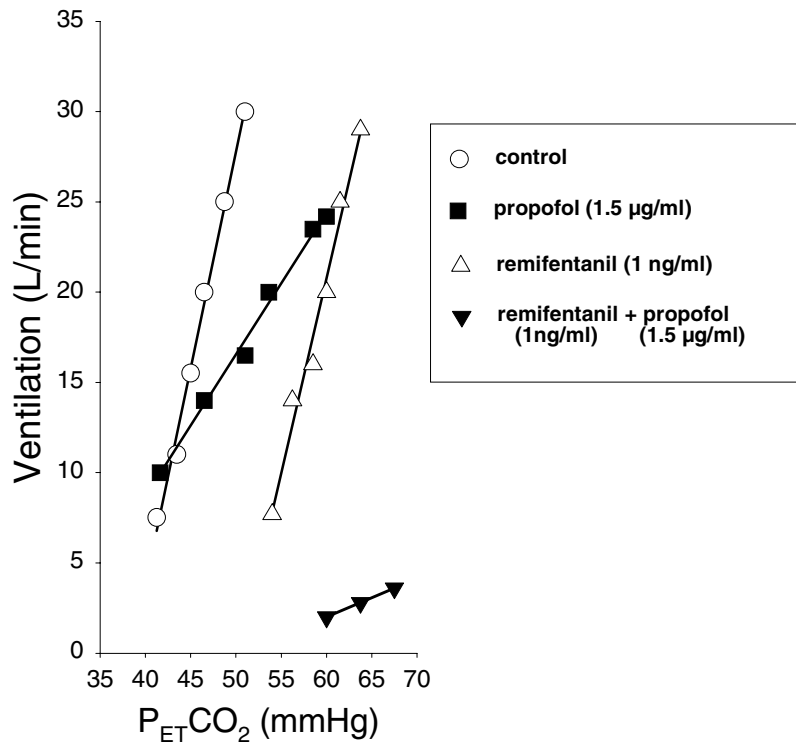


Figure 2. Four ventilatory carbon dioxide response curves of one subject. The control response had a slope of 2.4 L/min per mmHg. While propofol decreased the slope to 0.8 L/min per mmHg, remifentanil caused a parallel shift to higher PCO_2 values of about 12 mmHg (slope = 2.2 L/min per mmHg). The combined administration yielded both a reduction in slope of the response curve (slope = 0.2 L/min per mmHg) and a rightward shift of about 20 mmHg. These observations suggest synergy on the slope of hypercapnic response and ventilation at a fixed $P_{ET}CO_2$.

(i.e., estimate $\pm 1.96 \cdot SE$) and the bootstrap BC_a (biascorrected and accelerated) method.⁶⁹

RESULTS

All 22 subjects completed the protocol without major side effects. A total of 94 responses were obtained at different drug combinations. The range of the measured arterial remifentanil was 0.2 ng/ml. For propofol all measured concentrations were in the range of 0.2–0.6 $\mu\text{g/ml}$ except one (2.6 $\mu\text{g/ml}$). Consequently $C_{h,r}$ and $C_{h,p}$ were set to 1 ng/ml and 1 $\mu\text{g/ml}$, respectively, in the pharmacodynamic model

A typical example of respiratory studies in one subject is given in figure 2. It shows the control response (no drugs given) with a slope of 2.4 L min^{-1} mmHg^{-1} , the effects of 1.5 $\mu\text{g/ml}$ propofol (a 66% reduction of the slope of the $\dot{V}_T\text{-}CO_2$ response to 0.8 L min^{-1} mmHg^{-1}) and 1 ng/ml remifentanil (a parallel shift of the response curve with a slope of 2.2 L \cdot min^{-1} \cdot mmHg^{-1}) alone, and the effect of that drug combination, which was greater than the sum of the effects of either drug alone (a > 90% depression of the

slope to $0.2 \text{ L min}^{-1} \text{ mmHg}^{-1}$).

In table 1 the results of the bootstrap based model selection are given. For all respiratory parameters model 7 was best fitted to analyze the data (*i.e.*, non-linear relationship between drugs and effect, synergistic interaction, $Q_{\max} = 0.5$, fig. 1). The population estimates \pm SE and 95% confidence intervals, as derived from the NONMEM analysis, of the response surfaces are given in table 2 and for resting \dot{V}_i , resting $P_{ET}CO_2$, \dot{V}_{55} and S in figures 3 and 4. At 1 ng/ml and 1 $\mu\text{g/ml}$, remifentanil and propofol caused $\sim 28\%$ and 13% depression of resting ventilation, respectively. Combining propofol and remifentanil at these same blood concentrations caused 58% depression (eqn. 4), indicating the synergistic nature of the interaction. Similar observations were made for resting $P_{ET}CO_2$, \dot{V}_{55} and S , although the synergistic interaction strength was less (I_{\max} resting $\dot{V}_i = 1.9$ versus I_{\max} resting $P_{ET}CO_2$, \dot{V}_{55} and $S = 1.2-1.3$). At the combined infusion of 1 $\mu\text{g/ml}$ propofol and 1 ng/ml remifentanil the depression of \dot{V}_{55} was 82% (eqn. 4); the corresponding values for resting $P_{ET}CO_2$ and S were 23% and 69%, respectively.

To get an indication of the spread of data points over the surface and of the goodness of fit, we give bubble plots which show the distance of individual measured data points from the population surface (*i.e.*, residuals; figs. 3-5). These plots show evenly spread data over the tested dose ranges and the absence of overt misfits. The values of baseline MAP and HR indicate that the subjects were free of agitation or stress during the studies (table 2). The effects remifentanil and propofol on MAP and HR rate were not as remarkable as their effects on the respiratory parameters: depression at 1 ng/ml remifentanil and 1 $\mu\text{g/ml}$ propofol ranged from 4 to 12% (table 2). The effect of the combination was expected from the concentration-response curve of the individual agents (*i.e.* additive interaction or $I_{\max} = 1$, linear dose-effect relationship for MAP, non-linear relationship for HR, table 1).

The BIS was unable to unearth any sedative effect of remifentanil over the dose range studied by us (inert interaction, fig. 5). Furthermore, the effect of propofol on the BIS was independent of the remifentanil concentration. The propofol-BIS relationship was linear with 19% depression of the BIS at 1 $\mu\text{g/ml}$ plasma level.

DISCUSSION

The main findings of our study are as follows: (1) Over the dose range tested, remifentanil (0-2 ng/ml) and propofol (0-2.6 $\mu\text{g/ml}$) caused a dose dependent depression of respiration, as observed by an increase in resting $P_{ET}CO_2$ and decreases in resting \dot{V}_i , slope of the \dot{V}_i - CO_2 response and ventilation at a fixed $P_{ET}CO_2$ of 55 mmHg; (2) While remifentanil shifts the \dot{V}_i - CO_2 response curve in a parallel fashion to higher $P_{ET}CO_2$ levels, propofol reduces the slope of the response rather than shifting its position (pivot point at resting \dot{V}_i); (3) When combined, the depressant effect of propofol and remifentanil on resting \dot{V}_i , resting $P_{ET}CO_2$, S and \dot{V}_{55} is synergistic, with the greatest synergy observed for resting \dot{V}_i ; (3) The depressant effect of remifentanil and propofol on blood pressure and heart rate is modest, when given separately; when combined their depres-

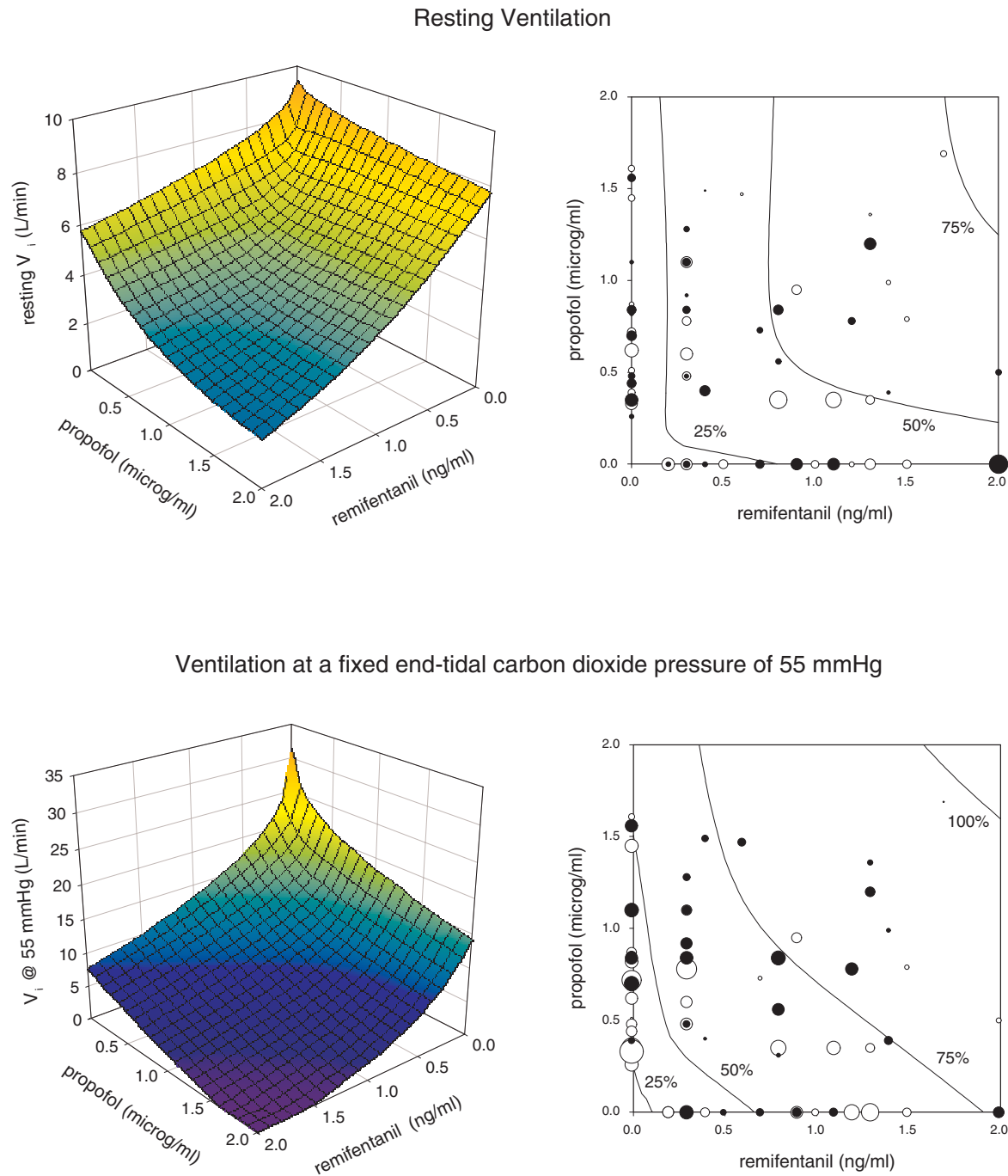
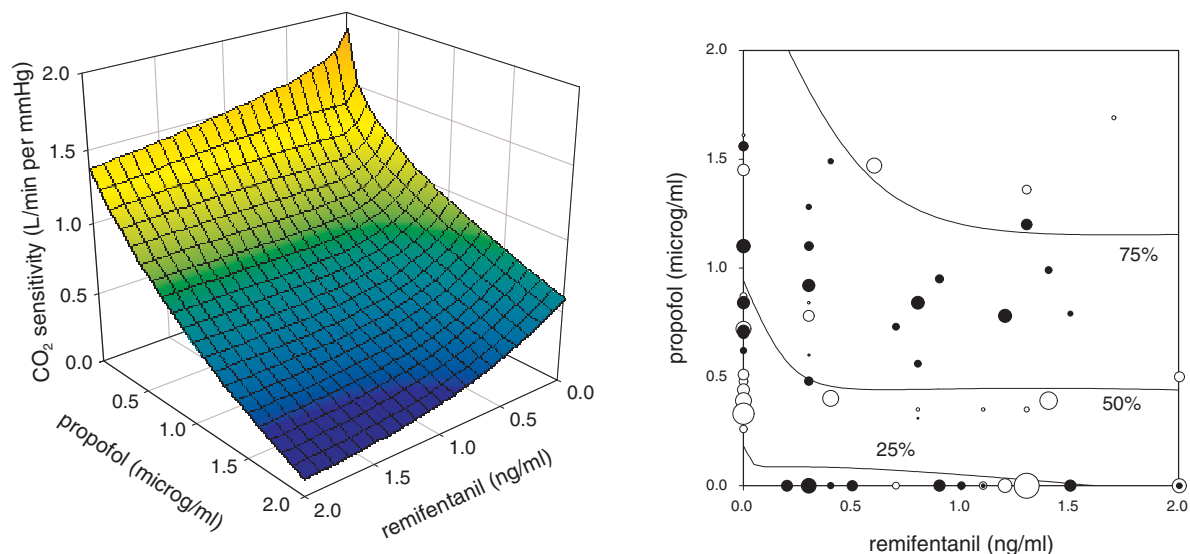


Figure 3. *TOP.* Left: Response surface modeling of the interaction of remifentanyl and propofol on resting \dot{V}_i . The population response surface shows that the propofol-remifentanyl interaction is synergistic ($I(Q) = 1.9 \pm 0.2$). Note further that the dose-response relationships between drugs and effect was not linear (for both drugs $\gamma = 0.5 \pm 0.1$). Right: Individual data points and 25, 50 and 75% isoboles. Open circles denote data point above the surface, closed circles below the surface (control data points not shown). The area of the circles is proportional to the distance from that data point to the surface. *BOTTOM.* Left: Response surface modeling of the interaction of remifentanyl and propofol on \dot{V}_i at a fixed $P_{ET}CO_2$ of 55 mmHg. The population response surface shows that the propofol-remifentanyl interaction is synergistic ($I(Q) = 1.2 \pm 0.1$). The dose-response relationships was not linear (for both drugs $\gamma = 0.4 \pm 0.1$). The model predicted apnea at several combinations of propofol and remifentanyl, e.g., 1.6 ng/ml remifentanyl and 2.0 μ g/ml propofol or 2.0 ng/ml remifentanyl and 1.6 μ g/ml propofol. Right: Individual data points and 25, 50, 75 and 100% isoboles.

Carbon dioxide sensitivity



Resting end-tidal carbon dioxide pressure

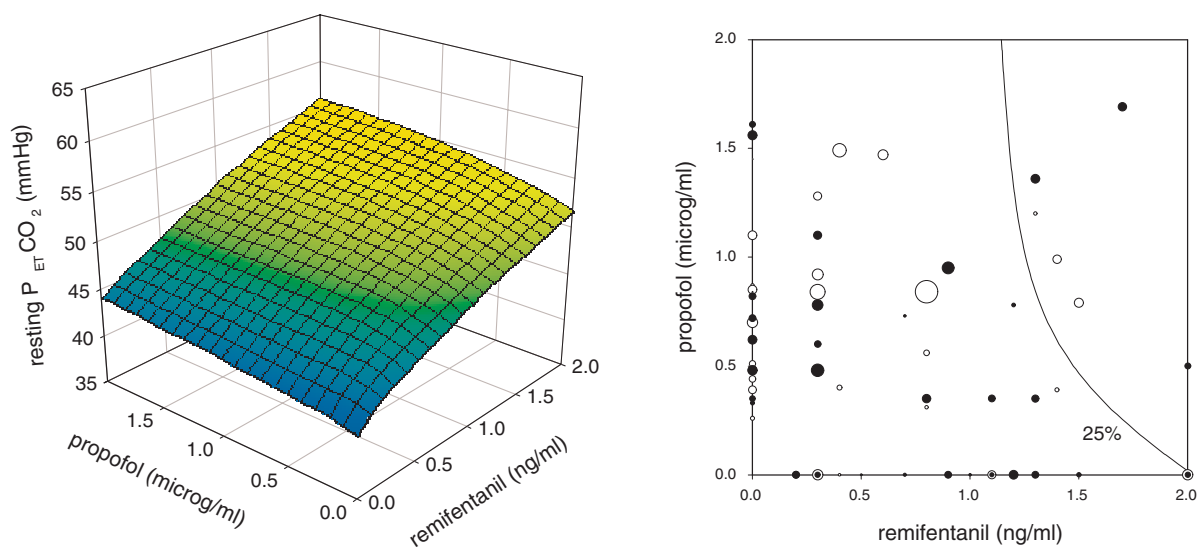


Figure 4. *TOP*. Left: Response surface modeling of the interaction of remifentanil and propofol on the slope of the \dot{V}_i response to CO₂ (CO₂ sensitivity). The population response surface shows that the propofol-remifentanil interaction is synergistic ($I(Q) = 1.3 \pm 0.1$). The dose-response relationships was not linear (for both drugs $\gamma = 0.4 \pm 0.1$). Note that the effect on slope was predominantly a propofol effect and to a lesser extend a remifentanil effect. Right: Individual data points and 25, 50 and 75% isoboles. Open circles denote data point above the surface, closed circles below the surface. The area of the circles is proportional to the distance from that data point to the surface area. *BOTTOM*. Left: Response surface modeling of the interaction of remifentanil and propofol on resting P_{ET} CO₂. The population response surface shows that the propofol-remifentanil interaction is synergistic ($I(Q) = 1.3 \pm 0.2$). The dose-response relationships was not linear (for both drugs $\gamma = 0.7 \pm 0.1$). Note that the x- and y-axes are different from the other response surface plots with the control point now facing the reader. Right: Individual data points and 25% isobole.

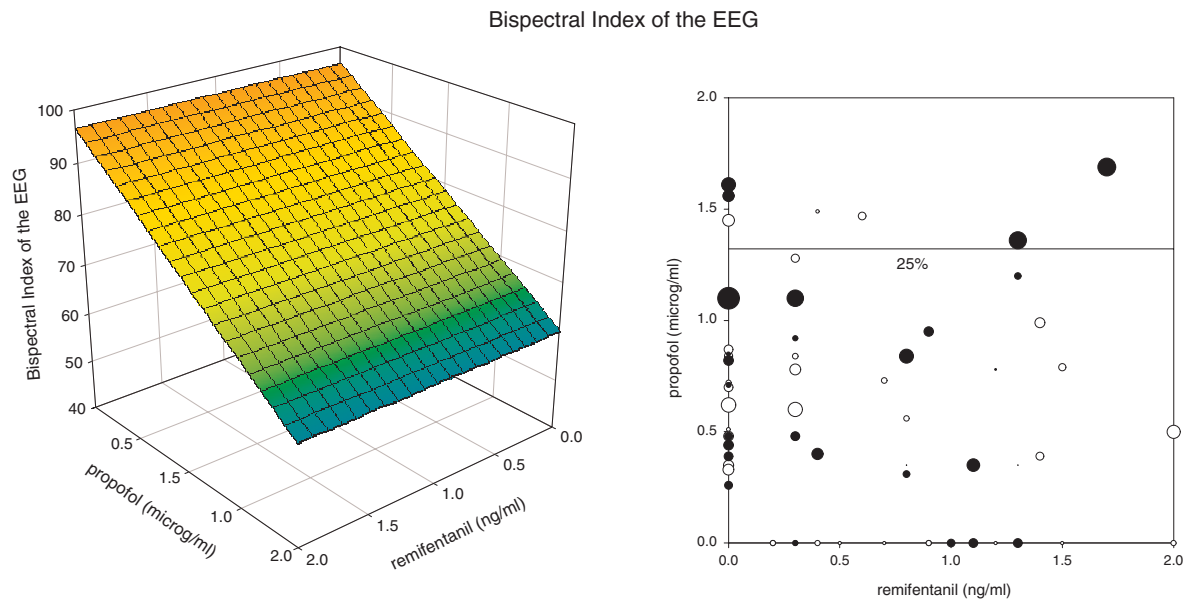


Figure 5. *LEFT:* Response surface modeling of the interaction of remifentanyl and propofol on the bispectral index of the EEG (BIS). The population response surface shows that the propofol-remifentanyl interaction is inert since remifentanyl had no effect on BIS irrespective of the propofol concentrations. Over this dose range, propofol causes a linear decrease in BIS with a 25% decrease occurring at 1.4 $\mu\text{g/ml}$. *RIGHT:* Individual data points and 25% isobole. Open circles denote data point above the surface, closed circles denote data points below the surface. The area of the circles is proportional to the distance from that data point to the surface area.

sant effect is additive; (4) The BIS is sensitive to propofol but not to remifentanyl, even when these agents are combined.

Pharmacodynamic Modeling

The pharmacodynamic model. In common with the study described in *Chapter 6* the pharmacodynamic model used by us is based on the 'Richards model' which for one drug is written as:¹⁵⁷ $f(x) = \alpha \cdot [(1 + \delta \cdot x^\gamma)^{1/\delta}]^{-1}$. By fixing $\delta = -1$ (*cf.* eqn. 1) a model is obtained which may be non-linear ($\gamma \neq 1$) or linear ($\gamma = 1$). The advantages of this approach have been discussed in *Chapter 6*. In short, in contrast to classical pharmacodynamic models, such as the inhibitory sigmoid E_{MAX} model, our model predicts apnea at and above certain drug concentrations; it predicts negative responses above certain drug concentrations;* and finally, linear respiratory dose-responses may occur over limited dose ranges.⁴⁸ Interaction was modeled as suggested by Minto *et al.*,¹²⁹ which is based on the following two ideas: (1) the combination of two drugs should be regarded as one new drug with its own properties, and (2) that these properties depend only on the concentration ratio Q . As before, interaction was defined by the function $I(Q)$, for which we chose a spline (for details see *Chapter 6*). Furthermore, the two drugs used in this study have dissimilar mechanisms of action so that we would not

*Negative responses may occur when testing the effect of opioids on the ventilatory response to hypoxia. See ref. 170 and *Chapter 6*.

expect their γ to be equal at equipotent concentrations. Therefore we also included the possibility of a linear $\gamma(Q)$. To our surprise $\gamma_r = \gamma_p = \gamma$ for all tested parameters.

Parameterization. Frequently, pharmacodynamic models incorporate C_{50} 's to describe and compare potencies. Since in our study the applied concentration ranges lie well below the C_{50} 's, these parameters are poorly estimated with wide and asymmetric confidence intervals. In order to overcome this problem we introduced the parameter λ which is the percentage depression at the concentration midway in the plasma concentration range (see eqn. 4).

Bootstrap Model Selection. The method of the bootstrap was applied here to assess the stability of the model selection based on *AIC*. Confidence in a model is then expressed as the number of times a model is chosen. Note that this confidence is not equivalent with the type I or type II error in traditional hypothesis testing. In the space of two nested models, however, the *AIC* is closely related to the type I error and the model selection percentage closely related to the power of the test.¹⁷⁸ When NONMEM produced an error message concerning boundary errors, the model that was tested was most probably overparameterized and would not be selected by *AIC* anyway.

Characteristics of Parameter Distributions. Parameter distributions can be estimated by constructing histograms of the estimated parameter values from the bootstrap runs. With the parameterization utilizing λ 's, their distributions were neither wide nor skewed so that the confidence intervals (obtained from the NONMEM population estimates $\pm 1.96 \cdot \text{SE}$, table 2) turned out to be equivalent with those obtained from the bootstrap parameter distributions. For example, for \dot{V}_{55} the corresponding values are baseline value 29.0–34.0 L/min, λ_r 51.0–67.0%, λ_p 37.0–52.0%, I_{\max} 1.08–1.39 and γ 0.22–0.50.

Parameter Values

The effects of 1 ng/ml remifentanil and 1 $\mu\text{g/ml}$ propofol on resting \dot{V}_i was considerably less than their effect on \dot{V}_{55} (the ratio of λ 's is 0.5 for remifentanil and 0.3 for propofol). This is not surprising taking into account the fact that while resting \dot{V}_i is measured under closed-loop conditions and part of the respiratory depression is offset by the gradual increase in resting $P_{ET}CO_2$, \dot{V}_{55} is measured under open-loop conditions and the pharmacokinetics and pharmacodynamic of CO_2 (and the effect the tested drugs have on CO_2 PK/PD) have been effectively removed. Recent studies indicate that C_{50} values obtained from studies using a fixed $P_{ET}CO_2$ input to the chemical control system and studies on the dynamic effect of drugs on resting ventilation, which do take into account the dynamics and kinetics of carbon dioxide, were of the same order of magnitude.²² For example, the C_{50} of alfentanil for depression of ventilation at a raised fixed $P_{ET}CO_2$ is about 75 ng/ml,[†] while the C_{50} derived from resting ventilation (i.e., without any inspired CO_2) is 60 ng/ml.²²

The extrapolated C_{50} values from this study correspond well with studies from the literature. For example, the remifentanil C_{50} of \dot{V}_i at a raised and fixed $P_{ET}CO_2$ obtained from a single bolus of 0.5 $\mu\text{g/kg}$ was of the same order of magnitude as our observa-

[†]see Chapter 6

tion (1.1 ng/ml versus 0.7 ng/ml in this study, table 2).⁴ Note that in this latter study remifentanil concentrations were not measured but obtained from the literature. These C_{50} values are a factor of 10 smaller than those observed for changes in spectral edge frequency of the EEG,¹²⁸ and 4 to 5 times smaller than those observed for 50% probability of adequate anesthesia during abdominal surgery (in combination with 66% nitrous oxide).⁶⁵ These findings indicate the higher opioid sensitivity of CNS sites involved in ventilatory control compared to sites involved in behavioral state control and suppression of somatic and autonomic responses. Remifentanil is about 80–100 times more potent than alfentanil in depressing \dot{V}_{55} .[‡] At present we are unaware of any previous respiratory PK/PD data for propofol.

Clinical Considerations

While response surface modeling provides a compact mathematical formulation for describing the interactions of two (or more) drugs, it can be difficult to translate this surface into a clinically useful interpretation. The isoboles (figs. 3–5) provide a horizontal ‘cut’ through the response surfaces, however, vertical cuts through the response surfaces may provide a more useful clinical graph. While remifentanil and propofol are often given at the same time to patients, they are not mixed together and infused at a constant ratio. General clinical use is for both drugs to be given at a constant rate (resulting in a steady state with constant plasma levels) and then one of the drugs adjusted up as needed for additional analgesia/sedation or down if less respiratory depression is important. The parameters of the response surface for resting $P_{ET}CO_2$ and ventilation can be used to predict how these important clinical variables will change with changing infusion rates. Since $P_{ET}CO_2$ is the more easily clinically monitored variable, figure 6 shows how $P_{ET}CO_2$ changes with the infusion rates. In the top panel, the increase in $P_{ET}CO_2$ with changes in propofol plasma concentration at constant remifentanil levels is shown, while the bottom panel shows the same for constant propofol concentration and remifentanil is adjusted. The non-linear shape of the response surface results in marked differences between these two figures. Figure 6 predicts that the $P_{ET}CO_2$ increases regularly with increasing remifentanil with some potentiation by the addition of propofol. However, the amount of propofol added does not change the amount of depression until higher levels of remifentanil are reached. These curves predict that while remifentanil causes hypercapnia, once beyond an initial additional rise in $P_{ET}CO_2$ when the propofol is started, there is little further respiratory depression as the propofol plasma level is increased. These graphs indicate that it might be safer to titrate the propofol dose with a constant remifentanil background if more or less sedation is needed, since there should be little change in the amount of respiratory depression, but if less respiratory depression is required, then the remifentanil would need to be reduced.

The above applies best to patients who maintain their breathing during anesthesia. In order to extrapolate our findings to postoperative patients, we plotted in figure 7 the

[‡]see Chapter 6

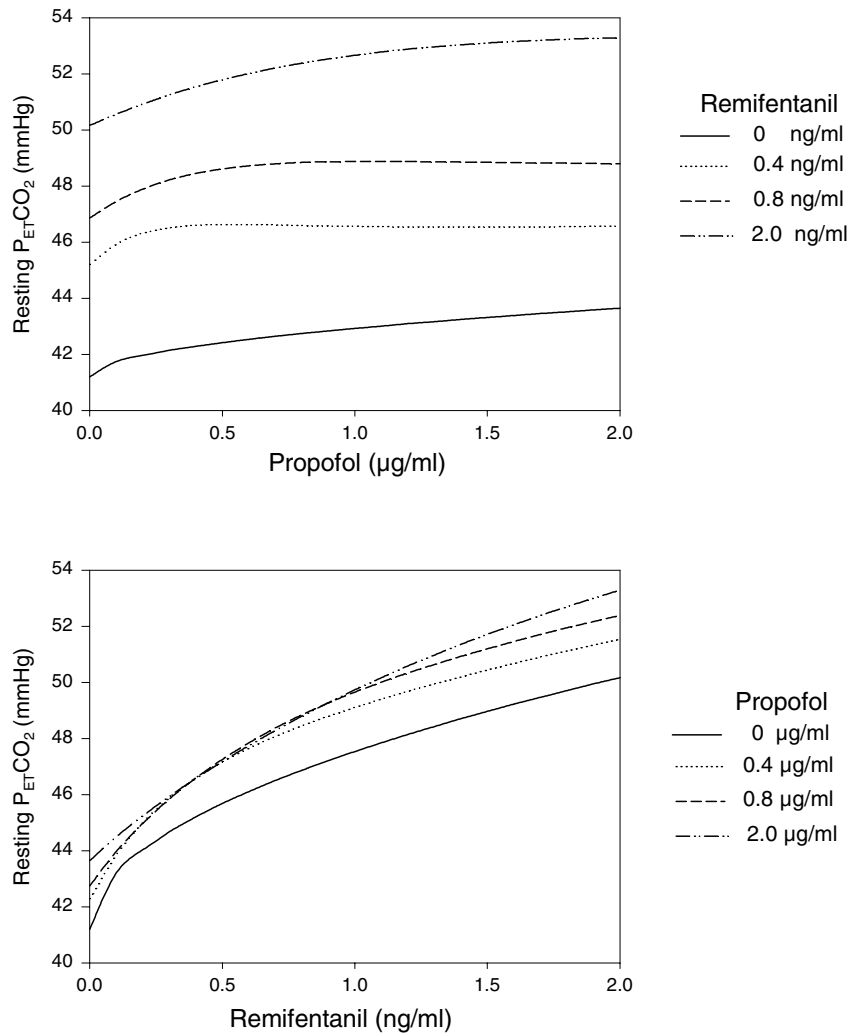


Figure 6. *TOP.* The influence of the steady-state or effect-site propofol concentration on resting $P_{ET}CO_2$ at various constant remifentanil concentrations. *BOTTOM.* The influence of the steady-state or effect-site remifentanil concentration on resting $P_{ET}CO_2$ at various constant propofol concentrations. Changing remifentanil concentrations causes marked increases in resting end-tidal PCO_2 , irrespective of the propofol concentration, while changes in propofol concentrations have less of an effect on resting $P_{ET}CO_2$, irrespective of the remifentanil concentrations.

10-60% isoboles of increasing resting $P_{ET}CO_2$ with the isobole for 50% probability of regaining consciousness after general anesthesia for abdominal surgery (and the isobole for 50% probability of no somatic/autonomic response to surgical stimuli).¹²⁵ This plot shows (1) the synergistic interaction between propofol and remifentanil on the 50% probability to 'wake-up' after anesthesia (and thus shows in contrast to the bispectral index data (fig. 5) the sedative/hypnotic effect of remifentanil); (2) whether consciousness has been regained or not, ventilation improves best by reducing the remifentanil concentration (*i.e.*, the return of the wakefulness drive is of limited importance at least

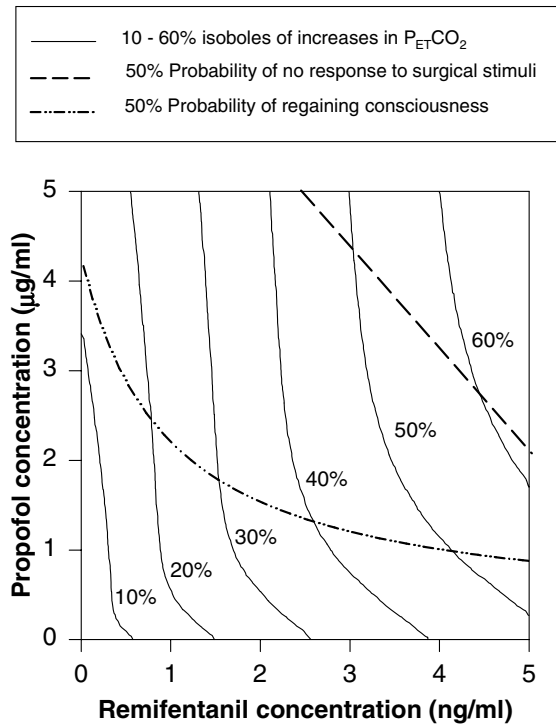


Figure 7. Comparison of isoboles of respiratory depression (10–60% isoboles for increases in $P_{ET}CO_2$, data from this study), consciousness and adequate anesthesia (50% probability lines for consciousness and adequate anesthesia in patients undergoing abdominal surgery, data from ref. 125).

when the subject is not stimulated or reminded to breathe); (3) without the addition of propofol, remifentanil concentrations up till 2 ng/ml cause only limited respiratory depression and may be applied for postoperative pain relief.

Because in our study ventilation and plasma drug levels were at steady state when data points were obtained we did not get information about the time-course of respiratory effects. Furthermore, especially for rapidly acting drugs, such as remifentanil and propofol, the degree of non-steady-state respiratory depression may be dependent on the rate of drug infusion. Further studies are needed to study the blood gas and \dot{V}_i dynamics caused by different infusion schemes of opioids and anesthetics.

Pharmacological Considerations

In this study we tested two agents with distinct respiratory properties and mechanisms of action. The opioid remifentanil caused a parallel shift of the \dot{V}_i - CO_2 response towards higher PCO_2 values with little effect on the slope (fig. 2). On the other hand, the anesthetic/sedative propofol caused a reduction of the slope of the \dot{V}_i - CO_2 response curve (S) with little to no effect on the position of the curve at resting $P_{ET}CO_2$ values (fig. 2). We consider these effects typical respiratory effects of opioids and anesthetics/sedatives. The effect of the opioid is because of activation of μ -opioid receptors at sites involved

in ventilatory control (*e.g.*, the carotid bodies, the preBötzinger complex);⁴⁴ the effect of the anesthetic is most probably related to less specific mechanisms such as changes in the level of arousal/consciousness and consequently a reduction in input from sites in the CNS involved in behavioral-state control (*e.g.*, the cortex, brain stem reticular system) to the ventilatory control system in the brainstem. The observation that the slope of the \dot{V}_T - CO_2 response during sedation with propofol was reduced is in agreement with the finding that the slope of the morphine \dot{V}_T - CO_2 response is reduced by physiological sleep.⁷² Previously, we observed large differences in the effect of i.v. morphine on the slope of the \dot{V}_T - CO_2 response in men and women.^{43,171} with no effect of morphine on the slope in men but a large reduction in women. Taken into account the above, it would be appropriate to suggest that in our previous studies morphine produced greater sedation in women than in men and consequently greater effects on S in women. Indeed, in a recent study in which we assessed the effect of morphine's active metabolite, morphine-6-glucuronide (M6G), on the level of sedation using a numerical rating score, we found greater sedation in women than men while plasma M6G concentrations were equal (unpublished observation). Note however, that our suggestion do not exclude more fundamental sex differences in CNS responses to opioids such as sex differences in μ -opioid receptor density and affinity in regions involved in ventilatory control and pain response.¹⁷⁴

8 Respiratory depression by tramadol in the cat: involvement of opioid receptors?

A MAJOR ADVERSE effect of opioid analgesics is respiratory depression which is probably mediated by an effect on μ -opioid receptors.^{43,44,166} The analgesic effect of the centrally acting synthetic opioid tramadol is thought to be mediated through both an action on μ -opioid receptors and the inhibition of the reuptake of monoamines and/or stimulation of their release.^{63,64,153,154} The affinity, however, of tramadol at μ -opioid receptors is much (> 6000 times) lower than that of morphine,^{153,154,88} and this makes it a potentially interesting analgesic with minimal respiratory depression. Indeed several clinical studies have reported the absence of a significant respiratory depression by an analgesic dose of tramadol.^{18,71,92,99,127,193,194,205} Some other studies, however, indicate that under some circumstances tramadol may cause respiratory depression.^{8,160}

A frequently used method to assess the effects of agents on breathing is to measure respiratory frequency, tidal volume and/or oxygen saturation. A more sensitive method, however, to assess ventilatory control is the CO_2 response curve because by measuring CO_2 sensitivity and the apneic threshold (extrapolated x -intercept of the response curve) it is possible to anticipate a patient's ability to respond to sudden hypercapnic or hypoxic loads, *e.g.*, following an obstructive apnea. Few studies have used the CO_2 response to assess tramadol's effect on breathing. In patients without cardiorespiratory disease, Seitz *et al.*¹⁸⁰ found a dose-dependent decrease in CO_2 sensitivity and mouth occlusion pressure response after intravenous doses of 1 and 1.5 mg/kg, respectively. Using the technique of end-tidal CO_2 forcing (DEF), we recently found that in healthy volunteers 100 mg oral tramadol reduced the carbon dioxide sensitivity of the peripheral and central chemoreflex loops by about 30%, an effect that is similar to that of an about equal analgesic dose of morphine.¹³¹

Thus, it seems that tramadol, at clinical doses, may be able to cause respiratory depression. Whether this depressant effect is mediated by opioid and/or monoaminergic mechanisms is unknown. The aim of the present study was to examine if tramadol can cause a dose-dependent respiratory depression in the anesthetized cat. Furthermore, to investigate a possible opioid mechanism of action, we investigated whether naloxone could reverse and prevent a possible respiratory depression by tramadol.

METHODS

The experiments were performed after approval of the protocol by the Ethical Committee for Animal Experiments of the Leiden University Medical Center. Fifteen cats of either sex (body weight 2.6–5.0 kg) were sedated with 10 mg/kg ketamine hydrochloride. The animals were anaesthetized with gas containing 0.7–1.4 % sevoflurane and 30 % O_2 in nitrogen. The right femoral vein and artery were cannulated, and 20 mg/kg α -chloralose and 100 mg/kg urethan, were slowly administered intravenously and the volatile anaesthetic was withdrawn. About

one hour later, an infusion of an α -chloralose-urethan solution was started at a rate of 1.0–1.5 mg/kg per h α -chloralose and 5.0–7.5 mg/kg per h urethan. This regimen leads to conditions in which the level of anaesthesia is sufficient to suppress pain withdrawal reflexes but light enough to preserve the corneal reflex. The stability of the ventilatory parameters was studied at a previous occasion and they were found to be similar to those in awake animals, as indicated by the fact that they were stable over a period of at least 6 hours.^{77,197,204}

To measure inspiratory and expiratory flow, the trachea was cannulated and connected via a Fleisch no. 0 transducer (Fleisch, Lausanne, Switzerland), which was connected to a differential pressure transducer (Statham PM197, Los Angeles, USA). With the aid of three computer steered mass flow controllers (HiTec, Veenendaal, The Netherlands) a prescribed composition of the inspire from pure oxygen, carbon dioxide and nitrogen could be obtained. The in- and expiratory fractions of O_2 and CO_2 were measured with a Datex Multicap gas monitor (Datex-Engstrom, Helsinki, Finland). Rectal temperature was controlled within 1 °C in each cat and ranged between cats from 36.5 to 38.5 °C. Femoral arterial pressure was measured with a strain gauge transducer (Statham P23aC, Los Angeles, CA, USA). All signals were recorded on polygraphs, converted to digital values (sample frequency 100 Hz) and processed by a PC. All signals were stored on a breath-by-breath basis.

Study Design

Three groups of cats consisting of five animals each were studied.

Group 1: These animals received three doses of tramadol iv up to a cumulative dose of 4 mg/kg (two consecutive doses of 1 mg/kg followed by a final dose of 2 mg/kg). After each dose 2-3 DEF runs were performed (starting about 15 min after the infusions) to analyze the effects of the agent on respiratory control (see below). Finally, 0.1 mg/kg iv naloxone was administered to these animals and again two DEF-runs were performed and analyzed.

Group 2: In these animals we determined the effect of an initial treatment with naloxone (0.1 mg/kg, iv) by performing DEF runs both before and after its administration. Thereafter, a single dose of 4 mg/kg tramadol was given intravenously and during the next two hours DEF runs were performed each 15 min to analyze the respiratory effects.

Group 3: In these animals a similar protocol as in group 2 was followed but without the naloxone pretreatment.

The ventilatory response to CO_2 was studied with the dynamic end-tidal forcing technique (DEF). We applied the DEF technique by imposing step-wise changes in the end-tidal CO_2 tensions at a constant normoxic background ($P_{ET}O_2 \sim 15$ kPa). Each DEF-run started with a steady state period of about 2 minutes, in which the end-tidal PCO_2 was maintained about 0.1–0.2 kPa above the resting value. Thereafter, the $P_{ET}CO_2$ was elevated by about 1–1.5 kPa within one or two breaths, maintained at a constant level for about 7 min and then lowered to the previous value and kept constant for a further 7 min.

Data Analysis

The steady-state relation of inspiratory ventilation to $P_{ET}CO_2$ at constant $P_{ET}O_2$ can be described by:^{54,55}

$$\dot{V}_i = (S_P + S_C)(P_{ET}CO_2 - B_k)$$

where S_P is the carbon dioxide sensitivity of the peripheral chemoreflex loop, S_C the carbon dioxide sensitivity of the central chemoreflex loop, and B_k the apnoeic threshold or extrapolated

$P_{ET}CO_2$ at zero. The sum of S_P and S_C is the overall or total carbon dioxide sensitivity (G_T).

For the analysis of the dynamic response of ventilation to a step-wise change in $P_{ET}CO_2$ we used a two-compartment model:⁵⁴

$$\begin{aligned}\tau_c \frac{d}{dt} \dot{V}_c(t) + \dot{V}_c(t) &= S_c [P_{ET,CO_2}(t - T_c) - B_k] \\ \tau_p \frac{d}{dt} \dot{V}_p(t) + \dot{V}_p(t) &= S_p [P_{ET,CO_2}(t - T_p) - B_k]\end{aligned}$$

Where τ_p and τ_c are the time constants of the peripheral and central chemoreflex loops, respectively, $\dot{V}_c(t)$ and $\dot{V}_p(t)$ are the outputs of the central and peripheral chemoreflex loops. $P_{ET}CO_2(t - T_c)$ is the stimulus to the central chemoreflex loop delayed by the central transport delay time (T_c), $P_{ET}CO_2(t - T_p)$ the input to the peripheral chemoreflex loop delayed by the peripheral transport delay time (T_p).

To allow the time constant of the ventilatory on transient to be different from that of the off transient τ_C is written as:

$$\tau_C = x \cdot \tau_{ON} + (1 - x) \cdot \tau_{OFF}$$

τ_{ON} is the time constant of the ventilatory on transient, τ_{OFF} the time constant of the off transient, and $x = 1$ when $P_{ET}CO_2$ is high, while $x = 0$ when $P_{ET}CO_2$ is low. In most experiments a small drift in ventilation was present. We therefore included a drift term ($C \cdot t$) in our model. The total ventilatory response, $\dot{V}_i(t)$, is made up of the contributions of the central and peripheral chemoreflex loops, the trend term and measurement noise (W):

$$\dot{V}_i(t) = \dot{V}_c(t) + \dot{V}_p(t) + C \cdot t + W(t)$$

The parameters of the model were estimated by fitting the model to the breath-by-breath data with a least-squares method. To obtain optimal time delays a 'grid search' was applied, and all combinations of T_p and T_c , with increments of 1 s and with $T_c \leq T_p$, were tried until a minimum in the residual sum of squares was obtained. The minimum time delay was chosen, arbitrarily, to be 1 s, the τ_p was somewhat arbitrarily constrained to be at least 0.3 s.

Statistical Analysis

Results are presented as means \pm SD. Differences between the obtained parameters in the control condition and after the three different doses of tramadol and after naloxone, respectively (group 1), were analyzed by performing a two way analysis of variance using a fixed model. The level of significance was set at 0.013. Control and naloxone data in group 2, and control and tramadol data in group 3 were compared with paired t -tests ($P = 0.05$).

Table 1. Respiratory variables from five animals obtained from the optimal model fits in the control conditions, after three cumulative iv doses of tramadol and after naloxone. Tramadol data were collected 15, 30 and 45 min after infusion and averaged. After naloxone, DEF runs were performed 15 and 30 min after administration and the obtained parameters from the optimal fits were averaged.

	control	1/mg/kg tramadol	2 mg/kg tramadol	4 mg/kg tramadol	0.1 mg/kg naloxone
No. of DEF runs	26	15	14	14	10
G_C	0.67 ± 0.27	0.45 ± 0.24	0.28 ± 0.17	0.21 ± 0.13	$0.69 \pm 0.13 *$
G_P	0.15 ± 0.04	$0.11 \pm 0.05 *$	0.05 ± 0.03	0.05 ± 0.03	$0.18 \pm 0.08 *$
G_T	0.82 ± 0.31	0.56 ± 0.29	0.34 ± 0.20	0.27 ± 0.16	$0.86 \pm 0.18 *$
G_P/G_C	0.27 ± 0.10	$0.27 \pm 0.06 *$	$0.21 \pm 0.07 *$	$0.28 \pm 0.10 *$	$0.26 \pm 0.09 *$
B_k (kPa)	3.77 ± 0.64	4.11 ± 0.86	4.31 ± 0.76	4.89 ± 0.95	$3.44 \pm 0.81 *$
MAP (mmHg)	134 ± 18	$136 \pm 13 *$	$131 \pm 13 *$	$125 \pm 15 *$	$130 \pm 30 *$

G_P , G_C and G_T : peripheral, central and total CO_2 sensitivity. Units $L \min^{-1} kPa^{-1}$;

MAP mean arterial pressure; Values are means of the individual mean values \pm SD;

* not significantly different from control. All other values are different vs. control at $P < 0.013$.

RESULTS

Examples of individual DEF runs in one animal from group 1 are shown in figure 1. In this example, 2 and 4 mg/kg tramadol reduced the CO_2 sensitivities of the peripheral and central chemoreflex loops and increased the apneic threshold indicating depressant effects on ventilatory output. The last panel in figure 1 shows that after infusion of 0.1 mg/kg naloxone these inhibiting effects were completely reversed. The results in all animals from group 1 are summarized in table 1. The total (= peripheral + central) CO_2 sensitivity in these animals (control value: $0.82 \pm 0.31 L \min^{-1} kPa^{-1}$) was reduced by 31, 59 and 68% by 1, 2 and 4 mg/kg tramadol, respectively, and these effects were caused by proportionally equal reductions in sensitivities of the peripheral and central chemoreflex loops (see unchanged G_P over G_C ratios in table 1). Also in a dose-dependent way, the apneic threshold increased from 3.77 ± 0.64 kPa in the control situation to 4.89 ± 0.95 kPa after the highest dose (P -values in legends of table 1). Lung-to-chemoreceptor time delays and time constants were not influenced by both agents (data not shown). After each tramadol dose we calculated the minute ventilation at a fixed PCO_2 of 6 kPa using the obtained values for the slope (G_T) and intercept (B_k) of the ventilatory CO_2 response curve. Figure 2 displays the dose-dependent decrease in minute ventilation at this PCO_2 level in the animals of group 1, ranging from a depression of about 45% after 1 mg/kg (mean $\dot{V}_{iTRAMADOL}/\dot{V}_{iCONTROL} = 0.55 \pm 0.16$) to about 84% after the total dose of 4 mg/kg (mean $\dot{V}_{iTRAMADOL}/\dot{V}_{iCONTROL} = 0.16 \pm 0.12$).

The last column in table 1 shows the mean results of two DEF runs recorded 15 and 30 min, respectively, after a final administration of 0.1 mg/kg naloxone to the animals of group 1. Control and naloxone parameter values did not differ from each

other indicating a complete reversal by naloxone of the depressant effects induced by tramadol. Neither tramadol nor naloxone caused significant changes in blood pressure (table 1). The reversal by naloxone of the tramadol-induced respiratory depression may indicate an action of tramadol on opioid receptors, and to investigate this further we investigated whether naloxone would be able to prevent respiratory depression from naloxone. Five animals (group 2) were pre-treated with 0.1 mg/kg naloxone (iv); 15 and 30 min later two DEF runs were performed and analyzed. The effects of naloxone on the respiratory variables in these five animals are shown in table 2. Thirty-five (35) min after the initial treatment with naloxone, a single dose of 4 mg/kg tramadol was administered and its respiratory effects were followed for two hours by performing and analyzing DEF runs each 15 min. To calculate \dot{V}_i at a fixed PCO_2 of 6 kPa at these time points, we used the optimal values obtained for the apneic threshold B and the CO_2 sensitivity G_T . Then, at these 15 min intervals, we determined the ratios of after and before the tramadol infusion at this fixed PCO_2 . The results are displayed in figure 2 (open symbols).

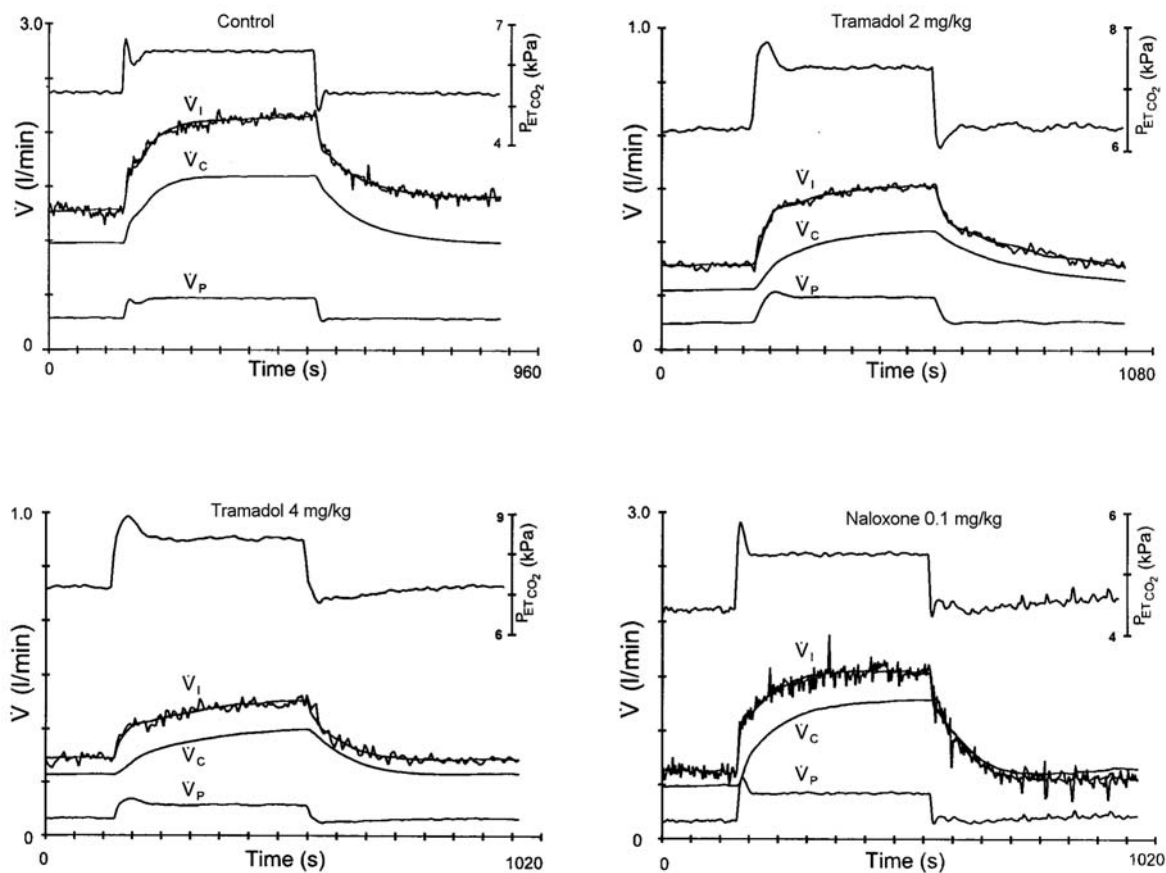


Figure 1. Examples of the DEF runs in one animal from group 1 showing the effects of control, 2 and 4 mg/kg tramadol, and (last panel) the total reversal of tramadol effect after naloxone (0.1 mg/kg) infusion. The top diagram in each panel is the P_{ETCO_2} input function. The line through the \dot{V}_i data is the sum of \dot{V}_C , \dot{V}_P and a trend term. \dot{V}_C and \dot{V}_P are the outputs of the central and peripheral chemoreflex loops, respectively.

Table 2. Effects of 0.1 mg/kg naloxone (iv) on mean (\pm SD) respiratory variables in five animals. Values after naloxone are means of two DEF runs performed 15 and 30 min after infusion.

	control	0.1 mg/kg naloxone
No. of DEF runs	22	10
G_C (L min ⁻¹ kPa ⁻¹)	0.41 \pm 0.12	0.57 \pm 0.44
G_P (L min ⁻¹ kPa ⁻¹)	0.09 \pm 0.05	0.10 \pm 0.06
G_T (L min ⁻¹ kPa ⁻¹)	0.50 \pm 0.17	0.67 \pm 0.49
G_P/G_C	0.20 \pm 0.08	0.20 \pm 0.12
B_k (kPa)	4.21 \pm 0.50	3.62 \pm 0.75 *

* significantly different from control at $P < 0.05$.

Five other cats (group 3) received the same single dose of tramadol but were not pretreated with naloxone (closed symbols in fig. 3). At each 15 min interval we calculated the ratios of after and before the tramadol infusion in the same way as in the animals of group 2. The effects of tramadol on the respiratory variables in these animals are shown in table 3. From figure 3 two findings are obvious. First, without naloxone pretreatment tramadol exerted its full depressant effect already 15 min after its administration (note that the data in table 3 show the means of all DEF runs performed in two hours). Second, after naloxone pretreatment the full depressant effect of tramadol developed much slower than without pretreatment, although naloxone did not prevent a rapid or subacute (as measured after 15 min) depression. The data in figure 3 strongly suggest that the increasingly depressant effect of tramadol in the naloxone pretreated animals over time are caused by a gradually declining effect of the opioid antagonist. At least part of the initial depression by tramadol (first data collection 15 min after tramadol *i.e.*, 15 min after naloxone, see fig. 3) may be caused by non-opioid mechanisms.

DISCUSSION

In this study we found that in the dose range of 1–4 mg/kg tramadol caused a dose-dependent depressant effect on ventilatory control consisting of a decrease in CO_2 sensitivity of the peripheral and central chemoreflex loops and an increase in the apneic threshold. In addition, in a dose of 0.1 mg/kg, naloxone completely reversed the depressant effect of a cumulative dose of 4 mg/kg tramadol, and prevented more than 50% of the depressant effect of an equal acute tramadol dose.

The reputation of tramadol as an analgesic lacking respiratory depression has contributed to its incremental clinical use in the intra- and postoperative period.⁵ The absence, however, of changes in end-tidal or arterial PCO_2 and/or ventilation does not preclude possible depressant effect on ventilatory control. Single respiratory variables such as respiratory frequency, tidal volume, oxygen saturation, etc. do not have any predictive value as to a patient's ability to respond adequately to hypercapnia and hy-

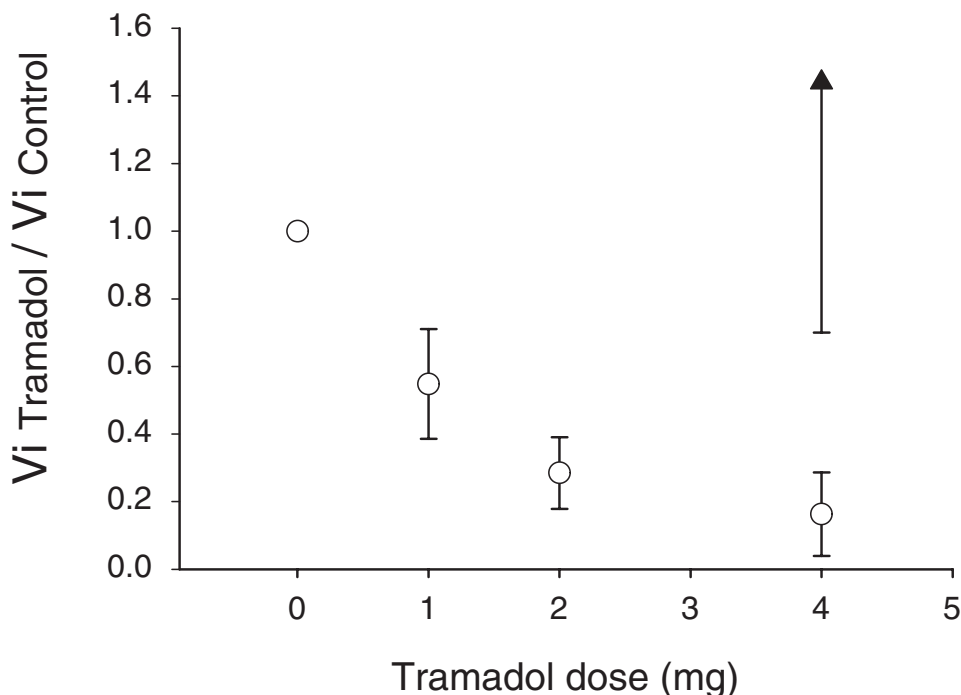


Figure 2. The dose-dependent decrease in relative ventilation at a fixed $P_{ET}CO_2$ of 6 kPa (\circ) in 5 animals. The triangle depicts the effect of naloxone given after 4 mg/kg tramadol.

poxia. Our data confirm and extend previous data obtained from humans,¹³¹ and show a clear dose-dependent increase in the apneic threshold and decrease in CO_2 sensitivity.

Our finding that the respiratory depressant effect of tramadol could be completely reversed by naloxone contrast with results obtained in clinical tests in which the opioid antagonist only partially inhibited tramadol's analgesic effect.³⁵ In humans, only about one-third of the antinociceptive action of tramadol, for which the parent compound is probably responsible can be reversed by naloxone.³⁵ The α_2 -adrenergic antagonist yohimbine, however, greatly reduced the antinociceptive action of 100 mg oral tramadol in healthy volunteers.^{59,60} In most animal tests, tramadol-induced antinociception was only partially reversed by naloxone.^{153,75} In contrast, intravenous yohimbine appeared to inhibit the antinociceptive effect of spinally administered tramadol but not morphine on the tail-flick response in the rat.¹⁵³ These results led to the hypothesis that the analgesic effect of tramadol is produced by both opioid and non-opioid *i.e.* monoaminergic mechanisms. The opioid effect may be mediated via μ -opioid receptors because tramadol's affinity at κ - and δ -opioid receptors is even lower than at the μ -receptor.^{153,88} Tramadol is a racemic mixture of two enantiomeres and the opioid action is exerted by the + enantiomer and its metabolite O-desmethyltramadol (M1) which has a greater affinity at the μ -receptor than its parent compound.¹⁵³ The monoaminergic mode of action may consist of an inhibition of the reuptake of serotonin and noradrenaline. This occurs mainly by the - enantiomer of tramadol and acts synergistically with the

Table 3. Effects of a single iv infusion of 4 mg/kg tramadol on respiratory variables in five animals. After tramadol, DEF runs were performed each 15 min during 2 hours.

	control	4 mg/kg tramadol
No. of DEF runs	22	37
G_C (L min ⁻¹ kPa ⁻¹)	0.59 ± 0.25	0.29 ± 0.11
G_P (L min ⁻¹ kPa ⁻¹)	0.14 ± 0.07	0.05 ± 0.03 *
G_T (L min ⁻¹ kPa ⁻¹)	0.73 ± 0.25	0.34 ± 0.12
G_P/G_C	0.27 ± 0.14	0.18 ± 0.13 *
B_k (kPa)	4.20 ± 0.37	4.77 ± 0.40

* not significantly different from control.

All other values $P < 0.05$ vs. control.

analgesic effect of the + enantiomer.^{7,30,63,64,88,153,154}

It is unknown whether respiratory depression by tramadol is also mediated via opioid and monoaminergic mechanisms. Our finding that naloxone completely reversed tramadol's depressant effects indicates an important contribution of opioid –probably μ -receptors, but does not necessarily imply that these effect were solely due to an opioid mechanism of action: we can not exclude that part of the relieve by naloxone from the tramadol-induced depression was caused by blockade of a tonic inhibitory influence of endogenous opioid peptides on ventilatory control in our animal preparation. For this reason we tested the effect of naloxone in a separate group of animals (group 2) without any pretreatment with tramadol; subsequently, these animals were given tramadol to see whether a respiratory depression developed. The ventilatory effects of these animals were then compared with those in animals receiving the same acute dose of tramadol but without being subjected to a pretreatment with naloxone. The finding that naloxone caused a moderate stimulatory effect on ventilatory control (an insignificant increase in CO_2 sensitivity and a significant decrease in the apneic threshold of ~ 0.6 kPa - table 2) indicates indeed a tonic inhibitory influence of endogenous opioid peptides in our animal preparation, which, however, is much too small to account for the very large stimulation that was seen in the animals in which the ventilation was greatly depressed by tramadol (table 1). Comparison of the respiratory behavior after tramadol infusion between animals with and without naloxone pretreatment (fig. 3) clearly shows that naloxone prevented more than 50% of tramadol's depressant effect. Figure 3 shows that tramadol exerts its effect rapidly: 15 min after administration the full depressant effect had already developed. We attribute the increasing respiratory depression with time in the naloxone-pretreated animals to a diminishing action of the opioid antagonist, which has a known half-time of ~ 90 min. By extrapolating the fitted curve in figure 3 back to the moment just after the tramadol infusion, we estimate that in our animals the pretreatment with naloxone prevented $\sim 70\%$ of tramadol's depressant effect. From these findings we conclude that, in contrast to its analgesic effects, the respiratory ef-

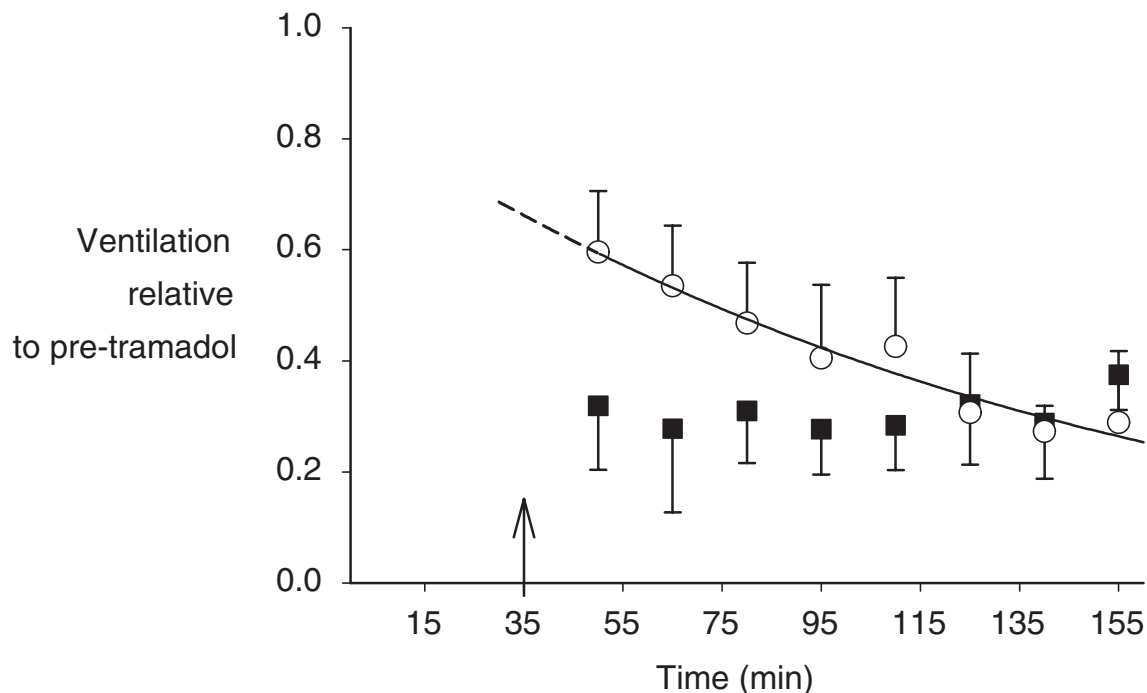


Figure 3. Open symbols: Influence of naloxone pretreatment (0.1 mg/kg, given at time=0) on ventilation at a fixed $P_{ET}CO_2$ of 6 kPa after infusion of 4 mg/kg tramadol (arrow) in five animals. Closed symbols: The same single dose of tramadol in five animals not pretreated with naloxone. The continuous line is an exponential function of time vs. ventilation relative to pre-tramadol in animals pretreated with naloxone from time $t = 50$ min on. The dashed line is an extrapolation to time $t = 35$ min (the time tramadol was given).

ffects of tramadol are mainly due to an action on opioid receptors. The remainder of the effect may be due to the inhibition of serotonin and/or noradrenaline reuptake (or by stimulation of their release), but experimental evidence for this is lacking. Generally, an increase in brain stem noradrenaline concentration has inhibitory effects on \dot{V}_i .³⁰ The effect of serotonin on ventilatory control is more complex and depends on the specific respiratory neuron and type of 5-HT receptor subtype involved.¹⁷

Because the effect of tramadol on the CO_2 sensitivity was caused by proportionally equal reductions in the sensitivities of the peripheral and central chemoreflex loops (unchanged ratio G_P/G_C - table 1) we suggest that the agent acts at the respiratory integrating centers within the brain stem, and in this respect tramadol does not differ from other agents acting at μ -opioid receptors. The effect of 1 mg/kg tramadol to reduce the CO_2 sensitivity by about 30% is about equal to that of 0.15 mg/kg morphine in the same animal preparation,¹⁶ indicating that as a respiratory depressant, in the cat morphine is 6-7 times more potent than tramadol. Although we are cautious to extrapolate our findings to men, the facts that in humans tramadol possesses about 1/6-1/10 of the analgesic potency of morphine,¹¹² an analgesic oral dose of 100 mg in healthy volunteers reduced the CO_2 response by about 30%,¹³¹ and intravenous doses

of 1 and 1.5 mg/kg clearly caused a decrease in CO_2 sensitivity,¹⁸⁰ indicate that in clinical doses tramadol may have similar respiratory depressant effects as we report here. Since in humans tramadol is often used in doses higher than 1 mg/kg, it would be useful to assess its possible depressant effect on the ventilatory CO_2 response curve and its reversibility by naloxone at these doses. In this way it could be anticipated whether a patient may be at increased risk during the occurrence of sleep apnea's or other events resulting in abnormal blood gas tensions.

SECTION 3

Postoperative Care

9 *The Edinburgh Ward 9 Study* or Ventilatory responses after major abdominal surgery and intensive care

IN THE FIRST few nights after major surgery, most patients have frequent episodes of airway obstruction and hypoxaemia.¹⁵⁶ These events cause cardiovascular responses which may contribute to the cardiovascular complications that occur at this time. The factors that cause these respiratory disturbances are not clear, but opioid analgesia, sleep deprivation, other centrally active medications, and the stress of major surgery may all play a part.^{3,85,101,120} We have recently shown that hypoxaemia can be reduced by oxygen therapy, but nasal continuous airway pressure, which might reduce episodic airway obstruction, does not improve either oxygenation or the quality of sleep in patients after major surgery.⁶⁶ Indeed we felt that such patients, even when judged 'fit to leave' the high dependency or intensive care unit, appeared not well recovered from the impact of surgery and the postoperative period, with breathing still depressed by postoperative analgesics. If respiratory responses are impaired, then an obstructive episode might persist longer, causing more profound and prolonged hypoxaemia.¹⁵²

In this study, we set out to assess how well patients after major abdominal surgery and intensive care were able to respond to episodes of airway obstruction, by simulating the changes in chemosensory input that they would experience during an episode of obstruction. To assess the response to obstruction, we devised a method to simulate the changes that would occur in the lung gases. We used a computer-controlled gas forcing system to increase CO_2 and reduce O_2 in the way these changes occur during obstruction. If the patient is in fact breathing clearly, then the changes in ventilation (\dot{V}_e) caused by this stimulus can be used as an index of the response the patient generates, although in a real obstructive episode increased breathing force would lead to increased muscle effort but not necessarily increased breathing. We also studied how the inflammatory response (indicated by the C-Reactive Protein (CRP) concentration), opioid medication, and opioid metabolites might be related to these responses.

METHODS

We studied respiratory responses to a combined hypoxic/hypercapnic stimulus in patients after abdominal surgery and 2 to 3 days intensive care (IC) in the high dependency unit (HDU) of the hospital (Ward 9 at the Royal Infirmary in Edinburgh; study period March - September 2000). Permission was obtained from the local ethics committee to recruit these patients before surgery and all subjects gave consent. Since the patients had to be moved to the respiratory laboratory, we were only allowed to do this when the patient was judged ready to leave the high dependency unit and return to the general surgical ward.

There were no restrictions in the use of drugs for induction and maintenance of general anesthesia. After surgery all patients were extubated. Initially, postoperative analgesia was

either epidural infusion of bupivacaine and morphine, or patient controlled intravenous analgesia (PCA) with morphine, according to the preferences of the attending anaesthetist. At the morning of discharge from the ICU all epidurals were removed and the patients set on PCA with morphine. All patients were asked to return for a review session 6 to 8 weeks after their discharge from the hospital.

Apparatus and Measurements

Patients were studied sitting in bed after discharge from the HDU in a semi-recumbent position, and in a comparable bed at their review visit. They breathed through a face mask (Vital Signs, Totowa, NJ) connected to a low resistance one way valve (Hans Rudolf model 2700). The exhaled gas from the valve passed through a heated pneumotachograph (Fleisch no. 2) and drying chambers to a dry gas meter (Parkinson Cowan CD4) modified to give a digital signal. This signal was used to calibrate the integrated expiratory flow signal and give an accurate breath by breath exhaled tidal volume. Gas was sampled at the mask and analyzed for O_2 and CO_2 concentrations by a mass spectrometer (VG Spectralab M, Winsford, UK) calibrated regularly with four standard gas mixtures. Breath by breath values for tidal volume (V_T), respiratory frequency (RR), instantaneous minute volume ($RR \times V_T$) and inspired and end-tidal partial pressures of O_2 and CO_2 were digitized (Dell 425 s/L computer, Dublin, Ireland) and stored on disc. The inspiratory side of the one-way breathing valve drew gases from a T piece with an open wide bore reservoir and a closed mixing compartment fed with O_2 , CO_2 and N_2 . These gases were delivered from mass flow controllers (Bronkhorst Hi-Tech, Veenendaal, The Netherlands) supplied with gas from precision regulators (RS components), and controlled by a computer (Elonex PT-5120/I) with a D to A converter (Amplicon PC24). This computer was supplied with data from the data acquisition computer. Custom written software calculated a rolling mean of the end-tidal O_2 and CO_2 from the last n breaths, where n could be adjusted between 1 and 10, and adjusted the mass flow controllers so that the inspired concentrations of the O_2 and CO_2 were the same as this mean end-tidal value. This caused a gradual decrease in inspired O_2 and concomitant increase in inspired CO_2 . The value of n was adjusted so that a fall in SpO_2 of 6% and an increase in end-tidal PCO_2 ($P_{ET}CO_2$) of 1 kPa occurred over 1-min. Sighing or swallowing cause sudden changes in the end-tidal concentrations, so breaths which differed from the target value by more than 5% were ignored. A pulse oximeter using an ear probe (Ohmeda 3700 set to an averaging time of 2 s, Ohmeda, Helsinki, Finland) and ECG (HP 78351A) were recorded throughout the study. We observed the patients carefully as well as monitored the EEG (A2000, Aspect Medical Systems, Newton, MA) for any evidence of sleep.

On both study occasions the patients first inhaled a normoxic gas mixture ($F_i = 0.21$) for at least 2 minutes until ventilation was stable. Next, one to three combined ramp-like hypoxic/hypercapnic stimuli (see above, duration 1-2 min) were administered. Subsequently the inspired oxygen fraction was increased ($F_i = 0.3$) and another one to three O_2/CO_2 stimuli (duration 1-2 min) applied. Each run was separated by at least two minutes of steady-state ventilation. If the baseline SpO_2 was less than 95%, the inspired oxygen was increased ($F_i > 0.3$) at the beginning of the study to increase the SpO_2 to a value $> 95\%$, and 3 to 6 stimuli were applied.

After the respiratory study was completed, venous blood was sampled and stored for assays of morphine (MOR), morphine-3-glucuronide (M3G), morphine 6 glucuronide (M6G) by HPLC,¹²¹ and for C-reactive protein (CRP) measurement using the FPIA method on an Abbott FLX appara-

Table 1. Patient characteristics, types of surgery and baseline parameters in the initially recruited group of 40 patients

Age (years)	66 (53, 68)	Heart rate (beats/min)	78	(67, 87)
Female/male ratio	16/24	-CRP (mg/dl)	17.6	(9.4, 24.0)
Type's of surgery:		-Morphine (nM)	34	(12, 86)
-Abdominal aorta reconstruction	10	-M6G (nM)	29	(19, 54)
-Bowel surgery	11	-M3G (nM)	526	(250, 767)
-Liver surgery	6	S_{pO_2} (%)	93	(92, 95)
-Whipple procedure	5	Ventilation (L/min)	9.6	(8.0, 11.1)
-Gastrectomy	4	RR (breaths/min)	15	(13, 19)
-Other abdominal procedures	3	V_T (ml)	613	(532, 762)
Length of stay in HDU (days)	2 (2, 3)	$P_{ET}CO_2$ (kPa)	5.3	(4.4, 6.3)
BIS	97 (95, 98)	$P_{ET}O_2$ (kPa)	13.3	(11.7, 15.7)

Values are median (25, 75 % quartiles); CRP, C-reactive protein; HDU, high dependency unit.

tus. Morphine and its metabolites were measured after leaving the high dependency unit only, CRP values were measured on both test occasions.

Data Analysis

Baseline parameters were averaged over one minute at the start of the measurements but prior to any change in inspired gas concentrations. The ventilatory response to the combined hypoxic/hypercapnic stimulus was analyzed using a non-parametric approach. Initially, we performed a linear regression of \dot{V}_e on $P_{ET}CO_2$ on the linear part of the \dot{V}_e - CO_2 response (as judged by the eye). Next, the resultant slope (G) was divided by the measured drop in S_{pO_2} . A delay in S_{pO_2} was taken into account because of the instrumentation and physiological delays.¹⁸² This yielded the value S . However, due to the fact that we applied ramps in inspired gas concentration (end-tidal gas values and S_{pO_2} were outcome parameters), especially in runs in which the inspired oxygen fraction was increased, no decrease in S_{pO_2} occurred or the decrease was 4 % or less. In these cases we decided not to divide G by the drop in S_{pO_2} and perform the statistical analysis on G rather than S .

Comparisons were made by non-parametric analysis (Kruskal-Wallis and Wilcoxon tests). Linear regression analysis was used to examine the relationships between variables (Sigmaplot 2001 for Windows, SPSS Inc.). P -values < 0.05 were considered significant. All values are expressed as median (25, 75 % quartiles).

RESULTS

A flow chart of the data acquisition and analysis is given in figure 1. Initially a total of 40 patients were recruited and tested (table 1). Only 26 patients returned for a second session (table 2). The ventilatory responses in seven of these 26 were so irregular or unreliable that an analysis of the data was not possible. In eight other subjects additional inspired oxygen was given prior to the application of the respiratory stimuli. Although all subjects completed the studies without major side effects, some of them did feel uncomfortable during the studies. We consider this the main reason for the

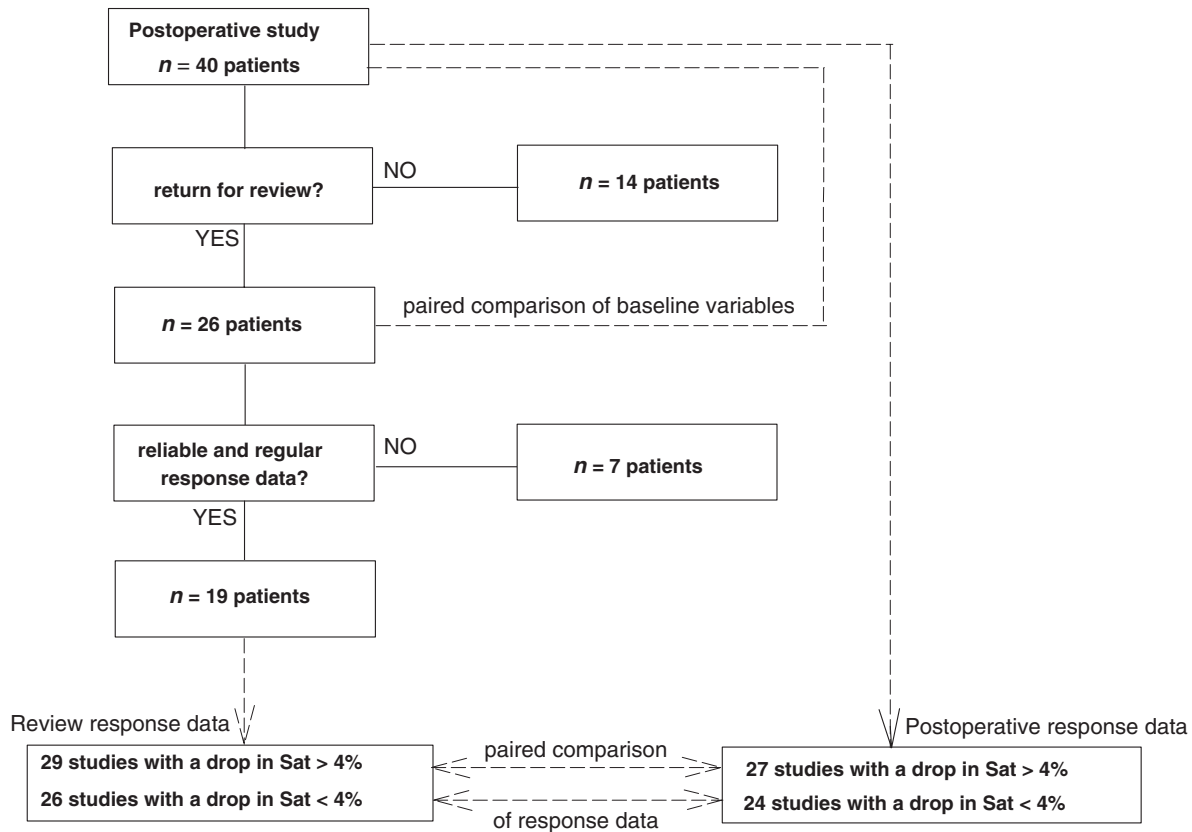


Figure 1. Flow chart of the data acquisition and data analysis of baseline variables and response data sets with and without drop in saturations.

rather disappointing return rate (26/40) and large number of curves that could not be analyzed (all curves in 7/26 patients). At the end of the study, response data from 19 patients were included in the analysis (48%).

In the initial studies, we noticed in none of the 40 subjects any subjective or EEG-related signs of sleep. Baseline parameters and blood concentrations of CRP, morphine and its metabolites are given in table 1. Note the large range of values (coefficients of variation were 78% (CRP), 149% (MOR), 70% (M3G) and 71% (M3G)). The variation in hospital $P_{ET}CO_2$ values but in none of the other variables could be partly explained by the plasma morphine concentration ($r^2 = 0.34$, $P < 0.05$). CRP, M6G and M3G values did not correlate significantly with any of the measured baseline variables.

After surgery and intensive care, patients showed evident signs of respiratory depression relative to the values obtained 6 to 8 weeks after hospital discharge (table 2), with greater $P_{ET}CO_2$ values, lower S_{pO_2} 's, lower tidal volumes and lower \dot{V}_e levels (18 patients had greater \dot{V}_e levels on review). Although respiratory rate did not differ between the two test occasions, analysis of the pattern of breathing revealed that the duration of inspiration was 1.3 (1.1, 1.5) s in postoperative patients, while on return for review this value was significantly longer (1.6 (1.5, 1.9) s, $P < 0.001$). The duration of expiration was similar in postoperative and review studies (postoperative 2.7 (1.9, 3.0) s *versus*

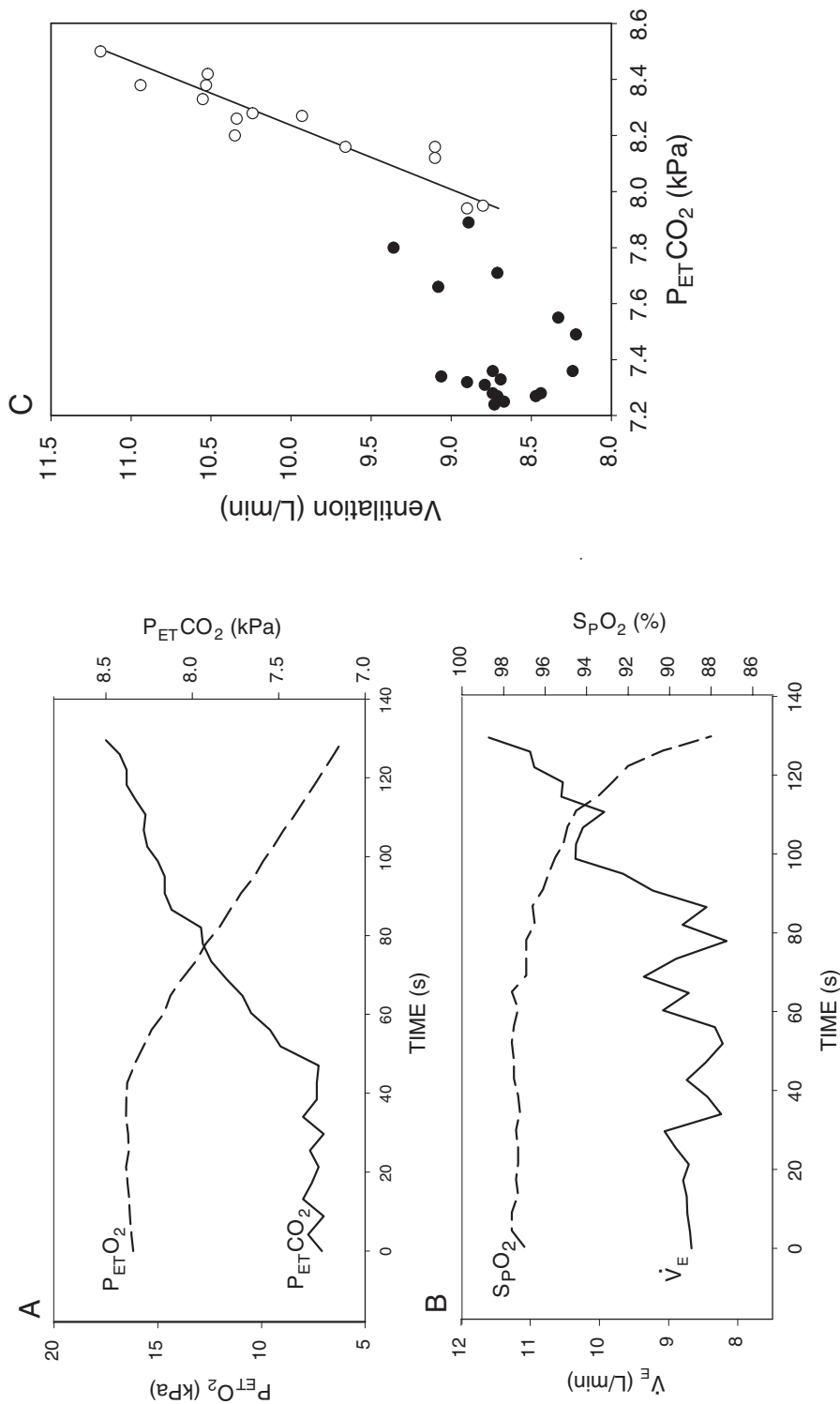


Figure 2. Example of the combined hypoxic/hypercapnic stimulus (A) and resultant changes in ventilation \dot{V}_e and O_2 -Hb saturation (S_{pO_2}) (B) of one subject. On the right (C), a plot of $P_{ET}CO_2$ versus \dot{V}_e and the linear regression on the linear part of the curve. Open symbols denote data points used in the regression analysis. The slope of the response curve (i.e., parameter G , which equals $4.4 \text{ L} \cdot \text{min}^{-1} \cdot \text{kPa}^{-1}$) was subsequently divided by the drop in S_{pO_2} (see diagram B) of 11% ($S = 0.4 \text{ L} \cdot \text{min}^{-1} \cdot \text{kPa}^{-1} \cdot \%^{-1}$).

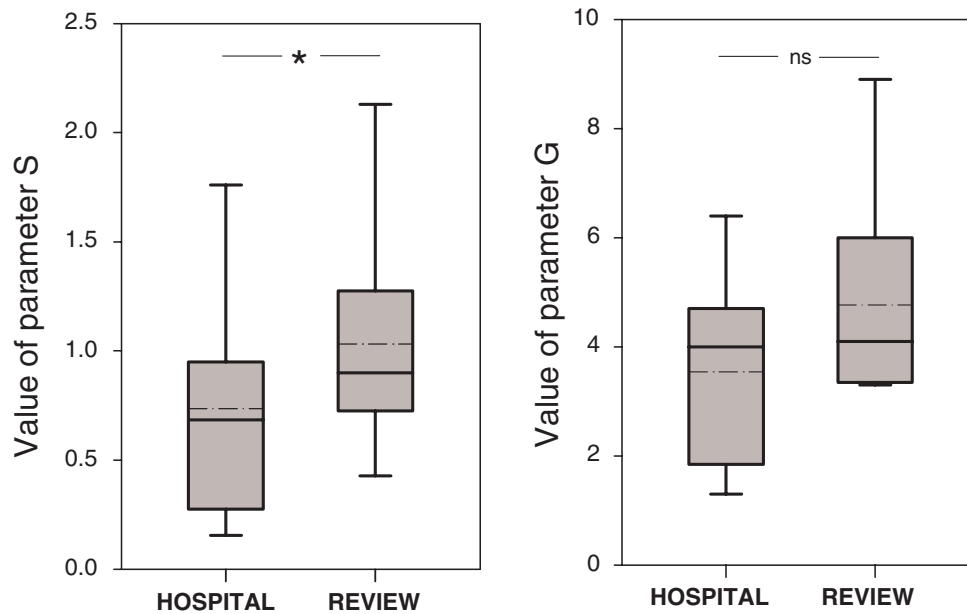


Figure 3. Box plots of the values of parameters S (left) and G (right). Values depicted are median (continuous lines), mean (dashed lines), 25 and 75% quartiles (top and bottom of boxes) and 10 and 90% quartiles (error bars) in data obtained postoperatively (hospital) and on review (6 to 8 weeks post hospital discharge) * $P < 0.02$; ns not significantly different (Kruskal-Wallis test).

review 2.4 (2.1, 2.8) s, ns).

An example of an O_2/CO_2 stimulus and resultant ventilatory response of one subject is given in figure 2. In 19 patients, a total of 27 responses with a fall in $S_pO_2 > 4\%$ and 24 with a fall $< 4\%$ were obtained after surgery and intensive care. On review the respective number of studies was 29 and 26. Values of the changes in S_pO_2 and $P_{ET}CO_2$ are given in table 3. On review, respiratory responses to an increase in CO_2 and decrease in S_pO_2 were about 25% greater than those obtained after surgery (S hospital = 0.69 (0.30, 0.85) $L \cdot \text{min}^{-1} \cdot \text{kPa}^{-1} \cdot \%^{-1}$ versus review = 0.90 (0.80, 1.20) $L \cdot \text{min}^{-1} \cdot \text{kPa}^{-1} \cdot \%^{-1}$, $P < 0.02$; fig. 3), while no difference was observed for the responses to an increase in CO_2 with little to no change in S_pO_2 (G hospital = 4.0 (1.9, 4.7) L/min per kPa versus review = 4.1 (3.4, 5.0) L/min per kPa, $P = 0.09$; fig. 3).

The distribution of values for C reactive protein in the ward and on return for review (table 2) show that the patients had a considerable inflammatory response at the time of discharge from the ward, which had returned to normal when they attended for review. We observed no correlation between CRP, morphine or its metabolites and degree of respiratory depression as determined from the ventilatory response to hypercapnia \pm hypoxia (ventilatory depression expressed as the proportion of the responses obtained at follow up (G(hospital)/G(review) and S(hospital)/S(review)).

Table 2. Hospital versus review baseline variables in 26 patients

	Hospital data		Review data		P-value*
CRP (mg/dl)	18.6	(7.0, 21.0)	0.9	(0.0, 2.2)	< 0.001
Morphine (nM)	32	(19, 84)	—		
M6G (nM)	29	(21, 54)	—		
M3G (nM)	580	(300, 760)	—		
BIS	96	(95, 98)	97	(95, 98)	ns
\dot{V}_e (L/min)	10.4	(8.0, 11.2)	10.6	(8.3, 12.6)	= 0.04
RR (breaths/min)	15	(13, 10)	16	(14, 18)	ns
V_T (ml)	629	(534, 817)	691	(611, 854)	< 0.05
$P_{ET}CO_2$ (kPa)	5.3	(4.0, 6.3)	4.7	(4.2, 5.1)	< 0.02
$P_{ET}O_2$ (kPa)	13.7	(11.9, 17.1)	14.1	(13.5, 14.7)	ns
S_pO_2 (%)	93	(92, 94)	97	(95, 98)	< 0.001
Heart rate (beats/min)	76	(66, 85)	74	(69, 88)	ns

Values are median (25, 75 % quartiles); * Student-*t*-tests; ns = not-significantly different; See legend of table 1 for explanation of abbreviations.

Table 3. Median values of the changes in oxygen saturation (S_pO_2) and end-tidal CO_2 concentrations ($P_{ET}CO_2$) observed at the end of O_2 and CO_2 ramps in hospital and review studies. Data are grouped according to the fall in S_pO_2 .

	<i>n</i>	ΔS_pO_2 (%)	$\Delta P_{ET}CO_2$ (kPa)
hospital $\Delta S_pO_2 > 4\%$	29	8.0 (6.1, 8.8)	0.8 (0.7, 1.0)
hospital $\Delta S_pO_2 < 4\%$	26	2.8 (1.4, 3.5)	1.0 (0.9, 1.1)
review $\Delta S_pO_2 > 4\%$	27	7.0 (5.0, 10.3)	1.1 (0.9, 1.3)
review $\Delta S_pO_2 < 4\%$	24	0.7 (0.5, 1.1)	1.0 (0.9, 1.1)

Values are median (25, 75 % quartiles). *n* is the number of runs.

DISCUSSION

In this study we compared respiratory variables and ventilatory pseudo-rebreathing responses in patients after abdominal surgery and intensive care and these same patients six to eight weeks after discharge from the hospital. The stimuli we used were intended to mimic changes in arterial oxygen saturation and CO_2 concentrations that occur once gas exchange has stopped due to upper airway obstruction. Due to the particular making of these stimuli (we used inspired control rather than end-tidal control of gas concentrations) a drop in saturation $> 4\%$ did not always occur, especially not when the study started with an increased inspired O_2 fraction. The oxygen reserve in the lungs and reduced oxygen metabolism are among the most likely causes for the absence in saturation fall in these patients during pseudo-rebreathing. Both kinds of stimuli do occur clinically in patients that develop upper airway obstruction for the same reasons as in our study. During upper airway obstruction, patients on oxygen de-

velop often high arterial CO_2 levels without any desaturations for many minutes, while patients that breathe air develop concomitant hypercapnia and hypoxemia.¹⁵²

Because of our study design (see fig. 1) each patient served as its own control. Initially but not on review, all patients showed elevated C-reactive protein levels, a sign of inflammatory response (table 2).⁸⁵ We observed that relative to review, respiratory depression was modest in postoperative patients: $P_{ET}CO_2$ was 11% greater, the ventilatory response to a CO_2 stimulus and drop in oxygen $> 4\%$ was about 25% reduced. No difference was observed in the ventilatory response to a CO_2 stimulus when saturation did not fall $> 4\%$, although an evident trend towards postoperative depression is visible in the data (fig. 3). While the low S_pO_2 value after surgery may be an indicator of reduced ventilatory drive, it must be remembered that oxygen saturation reflects the efficacy of gas exchange in the lungs and is not a measure of ventilation. The reduced values of S_pO_2 ($\sim 93\%$) may further be caused by atelectasis, a reduction in cardiac output, and/or increased oxygen metabolism.

The large number of patients that did not return for a review session and the relatively large number of data runs that could not be analyzed due to breathing instabilities may have under/overestimated the effect of surgery and analgesia on the response data. The median values of the O_2/CO_2 responses with and without fall in S_pO_2 in the 14 subjects that did not return for review were 0.61 (0.33, 1.1) $L \cdot \text{min}^{-1} \cdot \text{kPa}^{-1} \cdot \%^{-1}$ and 3.9 (2.4, 4.2) L/min per kPa, respectively. These values are not different from the 19 subjects that were included in the paired comparison. Hence we do not believe that the low return rate and sometimes poor data quality did influence our results significantly. Poor quality and increased variability of responses, relative to responses obtained in healthy volunteers, is inherent to the collection of clinical respiratory data. The large variability in the postoperative data may have been one of the reasons for not finding a significant effect on G between hospital and review (fig. 3). A *post-hoc* power analysis revealed that at this level of variability at least 30 patients were needed to get a significant 25% difference between studies.

Taken into account the complexity of the stimulus and resultant responses (see ref. 38 on the difficulties in the interpretation of CO_2 pseudo-rebreathing responses), we felt that it was necessary to develop a non-parametric method of describing the ventilatory response to the simultaneous hypoxic/hypercapnic stimulus. However, this non-parametric method will require some normalization by the level of the stimulus since the degree of hypercapnia and/or desaturation was not identical in all subjects and runs. In contrast to the gains of the peripheral and central chemoreflex loops as determined from single CO_2 steps (which represent the $\dot{V}_e\text{-}CO_2$ sensitivity of the peripheral and central chemoreceptors),^{36,38} one has to be cautious in assigning physiological to G or S in our analysis. However, since the drop in $S_pO_2 > 4\%$ at the background of mild hypercapnia must have stimulated the carotid bodies,⁴⁵ we believe that S in contrast to G incorporates respiratory responsiveness mediated *via* the peripheral chemoreflex loop (part of which may be $O_2\text{-}CO_2$ interaction at the carotid bodies).⁴⁵

We observed a difference in $P_{ET}CO_2$ of 0.6 kPa between the hospital and review visits. A similar value was observed in healthy volunteers who were without pain or inflamma-

tion and had similar estimated plasma levels of morphine.⁴³ We relate the increase in $P_{ET}CO_2$ in both studies to the respiratory depressant effect of morphine. Furthermore, in postoperative patients respiratory muscle function is often impaired due to a decrease in phrenic motoneuron output, which may be (vagal) reflex related to minimize irritation of the peritoneum after abdominal surgery.²¹³ This may be the cause of the reduced tidal volume we observed after surgery. The M6G levels were relatively low and most probably did not contribute to the respiratory effects of morphine. M6G concentrations > 300 nM are required for significant respiratory effect.* Our finding that S was depressed while G remained unaffected suggests that the peripheral chemoreflex loop was affected more than the central chemoreflex loop in our group of postoperative patients. This observation is in agreement with an earlier observation that the ventilatory response to a hypercapnic/hypoxic stimulus is affected at lower anesthetic and opioid concentrations than the response to a CO_2 stimulus when no fall in S_pO_2 is allowed.[†]

A Comparison with Volunteer Data

The depressant effect of morphine on respiration in healthy volunteers is well documented. For example, 0.1 mg/kg morphine bolus dose, followed by 30 μ g/kg per h continuous infusion (median steady-state morphine plasma levels estimated to be > 100 nM),¹⁷⁴ caused sex-dependent respiratory depression with about 50% depression of the ventilatory response to CO_2 steps in female volunteers and no effect on the step response in male volunteers but a parallel shift of the response slope to greater $P_{ET}CO_2$ values.⁴³ In another study, 0.07 and 0.20 mg/kg iv morphine depressed the ventilatory response to a ramp increase in $P_{ET}CO_2$ by 40% and 50%, respectively.²⁴ Although care has to be taken in comparing patient data with volunteer data and plasma morphine concentrations were considerably higher in some of the volunteer studies (see above), our results suggest that depression in postoperative patients on morphine was significantly less than depression observed in volunteers on morphine.^{24,43} This is not surprising taken into account the fact that respiration in perioperative patients is related to the balance between stimulation from pain, stress and activated chemoreflexes and depression resulting from sedation, the direct effect of analgesics and anaesthetics on respiratory neurones and the effect of surgery (see above). In our study depression of ventilatory responses occurred due to morphine (via its effect on μ -opioid receptors at central and peripheral sites),^{6,44,171} while stimulation of the responses may have occurred due to pain,^{21,23,172} stress, inflammation and discomfort. Interestingly, pain may both cause respiratory depression (patients that change their pattern of breathing to minimize diaphragmatic descent; the reduced inspiratory times may be the relection of such a mechanism (table 2)),²¹³ or stimulate breathing, often but not always *via* chemoreflex-independent mechanisms.^{21,23,172}

*Unpublished observation.

†See *Chapter 6*.

Clinical Considerations

Postoperative patients on opioid analgesics titrated to effect, seldom require intervention for apneic or periodic breathing. However, several studies showed frequent occurrence of upper airway obstruction and hypoxic episodes related and unrelated to upper airway obstruction in both awake and sleep states.^{61,66,155,156,189} We did not measure sleep-related breathing in our patients. But there is no reason to doubt that these patients did not experience upper similar airway patency problems and hypoxic events like those patients reported in the literature. Whether the mild to moderate depression of the ventilatory control system as observed in our group of patients plays some causative role in the occurrence of upper airway obstruction remains unknown but is plausible.¹⁵² A more important question is whether the ventilatory responses set at the restoration of blood gases in between episodes of upper airway obstruction remain sufficiently effective. The mild reduction in response data observed in our patients seems to suggest that this may indeed be the case. However, the ample data showing sometimes severe nocturnal hypoxemia after major abdominal surgery, which in some studies remains unaffected by oxygen or nasal continuous positive airway pressure therapy, suggests the contrary.^{66,189} Evidently, further studies on the effect of surgery and pain relief and their interaction on (hypoxia and CO_2 stimulated) breathing are required to improve our insight in this important part of postoperative patient care.

APPENDIX: A Modeling Approach to the Data Analysis

While a simultaneous ramp increase in $P_{ET}CO_2$ and a decrease in S_pO_2 provides a stimulus that is similar to clinical conditions (*e.g.*, airway partial obstruction), it does present some practical and theoretical problems with analysis. The physiological response to hypercapnia (at a constant saturation) is a linear increase in ventilation arising from both peripheral and central chemoreceptors.³⁶ As saturation is decreased (at a constant $P_{ET}CO_2$) there is also a linear increase in ventilation arising from stimulation of the peripheral chemoreceptors.⁴⁵ The interaction between saturation and CO_2 has been expressed as the change in the slope of the hypercapnic response for a decrease in saturation. The dynamics of both these response (hypoxic and hypercapnic) have generally been modeled with differential equations and given an appropriate input all these gains can be determined (along with the associate time delays and time constants).⁴⁰ With some reasonable assumptions, it is possible to modify our previously used two compartment dynamic CO_2 model used for parameter estimation and to obtain estimates of physiological parameters from this clinical data.

The relative slow changes in both the $P_{ET}CO_2$ and S_pO_2 does not provide an input that is ‘persistently exciting’ and the central and peripheral time constants cannot be estimated with any confidence. However, from past experience we can assume the peripheral time constant to be ~ 10 s and the central time constant to be ~ 120 s.⁴⁰ However, we can assume that the change in the saturation only effects the peripheral input. This allows us to modify our parameter estimation program to assume that the central and peripheral inputs are known signals (S_C and S_P) and the steady state ventilation is determined by a central and peripheral gain on these two signals:

$$\begin{aligned} S_C &= P_{ET}CO_2 \\ S_P &= (\alpha \cdot S_pO_2 + \beta) \cdot P_{ET}CO_2 \\ \dot{V}_i &= G_C \cdot (S_C - B_k) + G_P \cdot (S_P - B_k) + C \cdot t \end{aligned}$$

The saturation data needs to be time shifted (and interpolated) to match the breath-by-breath CO_2 and ventilation data. B_k is the so called apneic threshold and a trend term, C , is also included. These

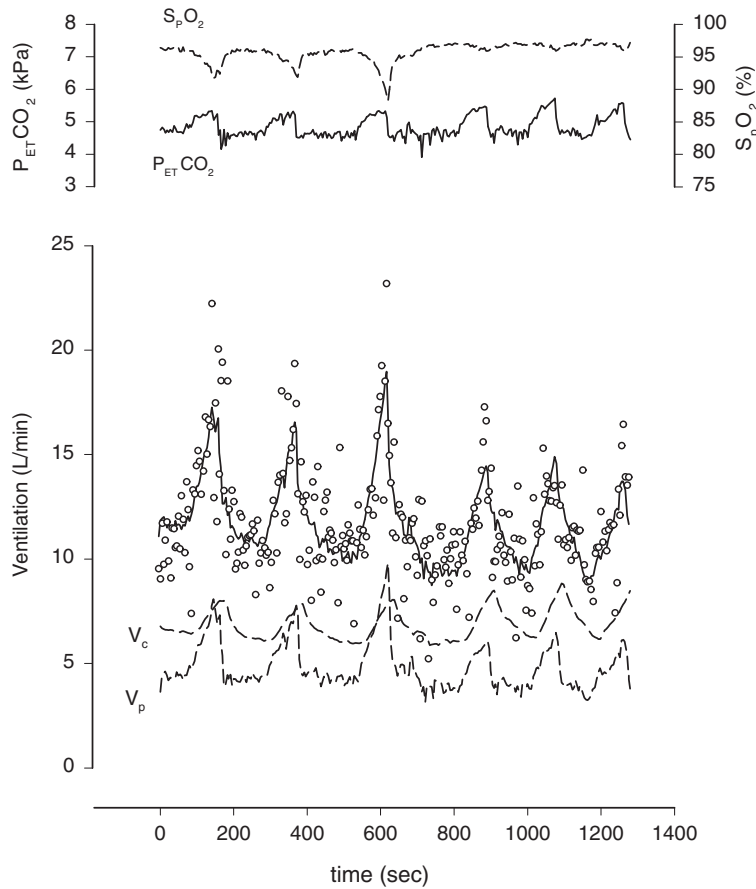


Figure 4. Example of a model fit in six sequential ramps in hypoxia/hypercapnia (initial three ramps) and hypercapnia. The data are from a patient returning for review. The upper panel shows the end-tidal CO₂ and saturation waveforms. The bottom panel shows the measured \dot{V}_e (open circles) and the model fit (the solid line going through the circles). The bottom two curves show the estimated central (\dot{V}_c) and peripheral (\dot{V}_p) components. Note that the peripheral component responds much faster and is altered by the level of saturation.

equations represent the steady state form of the model and in the parameter estimation program first order dynamics, with the assumed time constants given above, are included on each of the signals. The factors α and β are scaling factors that are chosen such that the value of the estimated G_p is of similar magnitude to that estimated in step hypercapnic experiments. The values chosen result in the hypoxic factor equal to 1 for 98% saturation and equal to 2 at 80% saturation. Note that these values are arbitrary in that they function only as scale factors and do not effect the final parameters estimated from the data set. Thus for this model the total CO₂ gain (change in ventilation for a change in CO₂ at 100% saturation) is:

$$G_T = G_C + G_P \cdot (100 \cdot \alpha + \beta)$$

and the percentage increase in the total gain for a 10% decrease in saturation:

$$\% \Delta G_T = \frac{10 \cdot \alpha}{G_T} \cdot 100\%$$

Figure 4 illustrates the curve fit of this model utilizing the whole data set which included 6 ramps, the first three are combined O₂/CO₂ stimuli, the last three hypercapnic stimuli. The data set was obtained

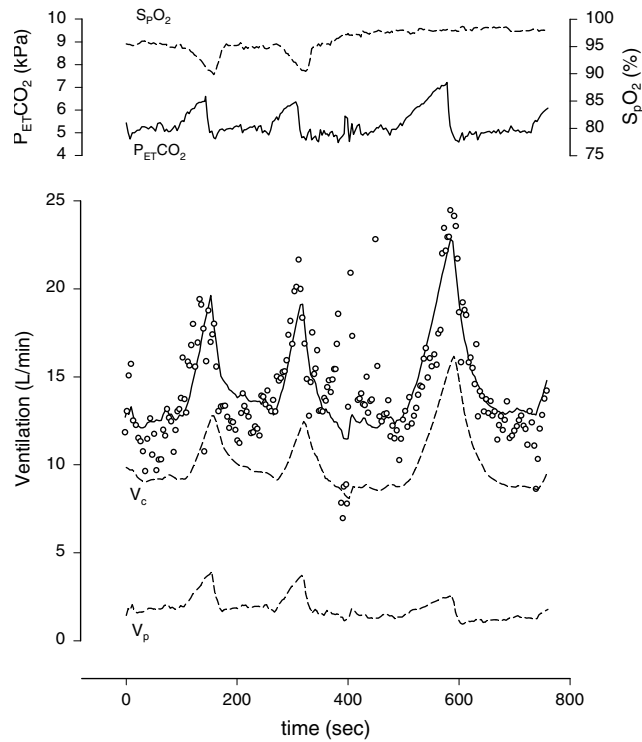


Figure 5. Example of a model fit in three sequential ramps in hypoxia/hypercapnia (first two ramps) and hypercapnia. The data are from a patient after surgery and intensive care. See Figure 4 for explanation of the symbols.

in a patient returning for review. Due to the absence of a hypoxic stimulus, the last three ramps elicit a much smaller peripheral signal. Note that the large increase in \dot{V}_e for the third ramp is apparently due to the deeper desaturation and the resulting larger peripheral component is seen in the figure. The parameters estimated for this data set was a total CO_2 gain of $7.05 \text{ L min}^{-1} \text{ kPa}^{-1}$ and a change in total hypercapnic gain with a 10% decrease in saturation of 21%. These values are comparable to those found in normal subjects.

Figure 5 illustrates another fit, this time to three ramps, two combined hypoxic/hypercapnic ramps and one hypercapnic one. This data set was obtained in a patient upon discharge from the high dependency unit. Note that for the third ramp the increase in CO_2 was greater than for the first two CO_2 ramps and even though there was no of desaturation, the resulting ventilatory increase was larger. This indicates that there is only a small amount of hypoxic-hypercapnic interaction. In fact, when this data set was curve fitted, we found that the total CO_2 gain was $7.15 \text{ L min}^{-1} \text{ kPa}^{-1}$ but the change in total gain with a 10% decrease in saturation was only 6%.

This dynamic parameter estimation technique requires a data set with little ventilatory instability or noise. As can be seen by the ventilation instability between the second and third ramps in figure 5, there can be significant ventilatory alterations that cannot be modeled as resulting from changes in CO_2 or saturation. Unfortunately, due to the clinical nature of our data sets, these types of ventilation changes were frequent and prevented us from applying this model to most of the data.

Summary and Conclusions

10 Summary and Conclusions

ANESTHESIA and surgery have profound effects on the control of breathing, which is best observed postoperatively. Ventilation is depressed, hypercapnia may occur, and recurrent hypoxic episodes are common in the first few postoperative days, especially when asleep. The patient may continue to breathe during a hypoxemic episode, but hypoxia and hypercapnia have further effects. They cause sympathetic nervous system activity, which can lead to tachycardia, hypertension and ischemic ECG changes. Afferent input from the peripheral chemoreceptor is an important stimulus to arousal, the clearing of upper airway obstruction and the subsequent hyperventilatory response to correct any hypoxia, hypercapnia and acidosis. Therefore it is of importance to understand the effect of anesthetics and analgesics on cardiorespiratory control and the mechanism of action of these agents. In this thesis, the results of experiments are described which improve our insight in the complex of factors that determine the cardiorespiratory control of perioperative patients. Studies were performed in animals, volunteers and patients.

In *Chapter 2*, the ventilatory responses to carbon dioxide (CO_2) and to hypoxia were examined in adult human subjects after bilateral and unilateral carotid body resection (CBR) for carotid body tumors, and compared with results obtained in (age-, and sex-matched) healthy volunteers. In humans, the ventilatory response to a step change in end-tidal CO_2 yields a fast ($\tau \sim 10$ s) and a slow component ($\tau \sim 120$ s). It is thought that the fast component arises from the peripheral chemoreceptors located in the carotid bodies. This study was an attempt to validate this hypothesis in humans. The results showed that in patients after bilateral CBR the ventilatory response to CO_2 was satisfactory fitted with a one-compartment model and an improvement of the fit was not obtained with a two-compartment model. When a significant improvement in fit did occur, with the introduction of a second fast component, it was associated with the presence of a peripheral chemoreflex response. This occurred in unilaterally CBR patients and control subjects. Our data validates the hypothesis that the fast component of the ventilatory response to CO_2 arises from the peripheral chemoreceptor at the carotid body.

In *Chapter 3*, we studied the effect of an antioxidant cocktail (ascorbic acid and α -tocopherol) on halothane-induced depression of the ventilatory response to brief exposures to hypoxia (3 min) in healthy volunteers. Halothane, at already subanesthetic concentrations (0.05–0.1 end-tidal %) causes profound depression of the carotid bodies and consequently of the ventilatory response to hypoxia. The precise mechanism of oxygen sensing at the carotid body remains elusive, but the involvement of free radical species has been proposed, and halothane, under hypoxic conditions, produces free radical species. The results showed that 0.1 MAC halothane-induced depression of the

hypoxic ventilatory response (halothane reduced the response by more than 50%), was completely prevented by pretreatment with the antioxidant cocktail, but not by placebo. This observation is important as it sheds light on the mechanism of oxygen sensing at the carotid bodies, improves our insight in the mechanism of halothane-induced depression of breathing at the carotid bodies, and, most importantly, opens new perspectives for therapeutic intervention to prevent potentially serious and life-threatening adverse effects in the postoperative period, following the use of inhalational anesthetics.

The influence of the intravenous anesthetic propofol on cardiorespiratory control was investigated in *Chapters 4 and 5*. Experiments, in healthy volunteers, were performed on the effect of propofol on various cardiorespiratory and EEG parameters, such as resting ventilation, resting end-tidal carbon dioxide partial pressure ($P_{ET}CO_2$), heart rate, bispectral index of the EEG (BIS), the acute and sustained hypoxic ventilatory response, and the dynamic ventilatory CO_2 response.

In *Chapter 4*, the results of the influence of low dose propofol (plasma concentration $0.6 \mu\text{g/ml}$) on the acute (3 min) and sustained (15 min) hypoxic ventilatory response are reported. The results showed that propofol decreased the acute hypoxic response (AHR) by $\sim 50\%$ and the ventilatory response to sustained hypoxia by $\sim 25\%$. Further, the magnitude of the slow ventilatory decline in ventilation from min 3 to min 15 of hypoxia was increased relative to the acute response by more than 50%. Interestingly, exposure to five consecutive 3-min hypoxic pulses did not generate any ventilatory decline in control or propofol studies. Since we did not perform CO_2 studies in this protocol, the mechanism behind the observed findings remain unclear: ventilatory depression due to propofol may be due an effect at peripheral or central sites involved in respiratory control or secondary to the sedation or hypnosis by propofol.

In *Chapter 5*, the possible site of action of propofol within the chemical ventilatory control system was investigated. We studied the influence of two concentrations of propofol (blood concentrations 0.5 and $1.3 \mu\text{g/ml}$) on the dynamic ventilatory response to CO_2 in healthy volunteers. The results showed that sedative concentrations of propofol (mean BIS 67) caused a dose related depression of the dynamic response to CO_2 which was attributed to an exclusive effect within the central chemoreflex loop. In other words, the peripheral chemoreflex loop remained unaffected by propofol. This is an important observation, and contrast sharply with the finding that all volatile anesthetics cause an selective depression of the CO_2 responses *via* the peripheral chemoreceptor. We relate these differences to the distinct mechanism by which the inhalational anesthetics and propofol act. It may very well be related to the antioxidant effect of propofol *versus* the prooxidant effect of inhalational anesthetics.

In *Chapters 6 and 7*, the effect of combining opioids and anesthetics on cardiorespiratory control is investigated. The combined administration of opioids and anesthetics for induction and maintenance of anesthesia (but also for Monitored Anesthesia Care and Conscious Sedation) is common practice. Therefore, knowledge on the quantitative and qualitative (additive *versus* synergistic) nature of their interaction is clinically

important and may lead to dosing regimens aimed at the titration of sedation or analgesic *versus* respiratory effect. The anesthetic-opioid interaction on cardiorespiratory control was assessed by response surface modeling. This approach enables us to construct three-dimensional representations of the concentration-response relation among infinite combinations of anesthetic and opioid concentrations and assess the nature of interaction (additive, synergistic, or antagonistic) over the whole surface area.

In *Chapter 6*, the influence of the combined administration of the inhalational anesthetic, sevoflurane, and the opioid, alfentanil, was investigated in healthy volunteers. The experiments consisted of step decreases in end-tidal partial pressure of oxygen from normoxia into hypoxia at constant $P_{ET}CO_2$. The experiments were performed at various concentrations of alfentanil and sevoflurane ranging from 0 to 50 ng/ml for alfentanil and from 0 to 0.4 end-tidal concentration (ET%) for sevoflurane, and at various combinations. The results were as follows: alfentanil and sevoflurane, when administered separately, depressed ventilation, HR, and the ventilatory and HR responses to acute hypoxia in a dose-dependent linear manner; when combined, their depressant effect on ventilation and HR was synergistic, whereas their effect on the hypoxic responses was additive; relative to normoxic baseline parameters (\dot{V}_i , V_T , RR , and HR) the responses to hypoxia showed greater sensitivity to the effects of alfentanil and sevoflurane (*i.e.* depression occurred at lower drug concentrations) when the drugs are administered separately and when combined; the BIS was sensitive to sevoflurane but not to alfentanil, even when these agents were combined (inert interaction).

In *Chapter 7*, the effect of steady state concentrations of intravenous anesthetic, propofol, and short acting opioid, remifentanil, given separately and in combination, on cardiorespiratory control and BIS was assessed in 22 healthy volunteers. In each subject one control, one remifentanil, one propofol, and at least one propofol-remifentanil combined study were obtained (measured arterial blood concentration range for propofol 0-2.6 $\mu\text{g/ml}$, and for remifentanil 0-2.0 ng/ml). Respiratory studies consisted of ventilatory responses to three to five increases in $P_{ET}CO_2$. The results show the following: remifentanil and propofol caused a dose dependent depression of respiration, as observed by an increase in resting $P_{ET}CO_2$ and decreases in resting ventilation, slope of the ventilatory response to CO_2 (S), and ventilation at a fixed $P_{ET}CO_2$ of 55 mmHg (\dot{V}_{55}); whereas remifentanil shifted the \dot{V}_i - CO_2 response curve in a parallel manner to higher $P_{ET}CO_2$ levels, propofol reduces the slope of the response rather than shifting its position; When combined, their depressant effect on resting \dot{V}_i , resting $P_{ET}CO_2$, S , and \dot{V}_{55} was synergistic, with the greatest synergy observed for resting \dot{V}_i ; the depressant effect of remifentanil and propofol, when administered separately, on blood pressure and heart rate was modest, when combined their depressant effect was additive; the BIS is sensitive to propofol but not to remifentanil, even when these agents are combined. Furthermore, these results indicate that in the clinical situation, when combining remifentanil and propofol in a spontaneous breathing patients, it might be safer to titrate the propofol dose with a constant remifentanil background if more or less sedation is needed, since there should be little change in the amount of respiratory depression, but if less respiratory depression is required, then remifentanil would need

to be reduced.

The influence of tramadol on ventilatory control was investigated in *Chapter 8*. Tramadol is an analgesic with putative opioid and non-opioid modes of action. The respiratory effects of tramadol are not clear, with studies showing conflicting results. The contribution of the μ -opioid receptor to tramadol induced respiratory depression, as measured by its effect on the \dot{V}_T - CO_2 -response, was determined in the anesthetized cat. Respiratory depression by tramadol was reduced by $\sim 70\%$ after naloxone pretreatment, indicating that at least 70% of tramadol's respiratory effect is related to its action at opioid receptors.

In *Chapter 9*, the complex of factors that interact on the cardiorespiratory control system in postoperative patients is examined. In this study we assessed how well patients after major abdominal surgery and intensive care were able to respond to episodes of airway obstruction, by simulating the changes in chemosensory input that they would experience during an episode of obstruction. We used a computer-controlled inspired gas forcing system to increase CO_2 and reduce O_2 in the way these changes occur during airway obstruction. The effect of 1 to 3 ramp-like combined hypoxic and hypercapnic stimuli, without initial added O_2 and 1 to 3 with initial increased inspired O_2 ($F_i = 0.3$) on ventilation was assessed in 40 patients after major abdominal surgery and 3 days of intensive care (IC) in a high dependency unit (Ward 9 of the former Royal Infirmary of the University of Edinburgh). Six to eight weeks after discharge from the hospital the patients were asked to return for a review study, and so each patient served as its own control. The results show that initially but not on review, all patients showed elevated C-reactive protein levels, a sign of inflammatory response. Furthermore, while morphine blood concentrations were relatively high (> 50 nM), respiratory depression was modest with only $\sim 30\%$ depression of ventilatory responses to the combined hypercapnic and hypoxic stimuli and 11% increase in resting $P_{ET}CO_2$. Our results suggest that depression seen in patients was about half of the depression seen after morphine in volunteers. This is not surprising taken into account the fact that respiration in patients is related to the balance between stimulation from pain, stress, the inflammatory response, and activated chemoreflexes and depression resulting from sedation, the direct effect of analgesics and anesthetics on respiratory neurones. This seems to suggest that the ventilatory responses set at the restoration of blood gases in between episodes of upper airway obstruction remain sufficiently effective.

In Conclusion, the data collected in this thesis show that:

- The fast component of the ventilatory response to CO_2 arises from the peripheral chemoreceptor at the carotid body;
- Halothane-induced depression of the ventilatory response to hypoxia is prevented by pretreatment with antioxidants;

- A sedative dose of propofol decreases the acute hypoxic ventilatory response by ~50%;
- Propofol, at a bispectral index value of 60 to 70, causes depression of the dynamic response to CO_2 via an exclusive effect within the central chemoreflex loop;
- Alfentanil and sevoflurane, when administered separately, depress ventilation, heart rate, and the ventilatory responses to acute hypoxia in a dose dependent linear manner, when combined, their depressant effect on ventilation and heart rate is synergistic, whereas their effect on the hypoxic responses is additive;
- Remifentanil and propofol cause, when administered separately, a dose dependent depression of respiration. When combined the depressant effect of remifentanil and propofol on resting \dot{V}_i , resting $P_{ET}CO_2$, S, and \dot{V}_{55} is synergistic, with the greatest synergy observed for resting \dot{V}_i . The depressant effect of remifentanil and propofol, when administered separately, on blood pressure and heart rate is modest, when combined their depressant effect is additive;
- The bispectral index is unable to extract the sedative effects of opioids, alone or in combination with anesthetics;
- Tramadol depresses the ventilatory response to CO_2 . This effect is at least for 70% related to its action at opioid receptors;
- In patients after major abdominal surgery and Intensive Care on PCA morphine, respiratory depression is modest and about half of what is seen after morphine in volunteers.

Valuable information regarding the cardiorespiratory control in perioperative patients in this thesis was obtained. The precise mechanism and complex of factors that play a role in perioperative respiratory depression remain unknown. Elucidation of these mechanisms is of evident clinical importance. Future studies should focus on the complex way the effects of genetic and acquired risk factors (*e.g.*, sex, congenital hypoventilation syndrome, obesity, sleep apnea syndrome, age), pain and pain relief, arousal state, upper airway obstruction, oxygen therapy, surgery and direct depressant effects of residual anesthetics on respiratory sensors and neurons interact and together determine respiratory efficacy (breathing and breathing responses to hypoxia and hypercapnia) in perioperative patients. A first attempt was made in the study described in *Chapter 9*. In this study we found a modest respiratory depression despite relative high morphine blood concentration. Suggesting that the ventilatory responses set at the restoration of blood gases in between episodes of upper airway obstruction remain sufficiently effective. However, the ample data showing sometimes severe nocturnal hypoxemia after abdominal surgery suggest the contrary. Evidently, further studies are required to resolve this matter.

10 Samenvatting en Conclusies

ZOWEL ANESTHESIE als de operatie beïnvloeden het ademregelsysteem. De effecten zijn in de eerste twee tot drie dagen na de operatie duidelijk aantoonbaar. In deze periode is het ademminuutvolume gereduceerd, er kan sprake zijn van hypercapnie en frequente en kortdurende perioden van soms diepe hypoxie komen voor. Dit laatste doet zich met name voor tijdens de slaap. Ook treden perioden van bovenste luchtweg-obstructie (obstructieve apneu's) frequent op. Tijdens een hypoxische periode blijft de patiënt ademen, maar hypoxie en hypercapnie hebben verdere effecten. Het sympathische zenuwstelsel wordt geactiveerd en dit kan weer leiden tot tachycardie, hypertensie en ischaemische ECG veranderingen. Om hypoxie, hypercapnie en acidose al dan niet veroorzaakt door bovenste luchtwegproblematiek te bestrijden zijn goed functionerende perifere chemoreceptoren noodzakelijk, niet alleen voor de noodzakelijke hyperventilatoire respons, maar ook om de bovenste luchtwegmusculatuur aan te spannen en aldus bovenste luchtwegobstructies op te heffen of te voorkomen. Het is daarom van belang om de interactie en het werkingsmechanisme van anesthetica en analgetica op het respiratoir regelsysteem te bestuderen en te begrijpen. In dit proefschrift worden experimenten beschreven die het inzicht in het complex van factoren die samen het (cardio)respiratoir regelsysteem bepalen en beïnvloeden bij perioperatieve patiënten, vergroot. De experimenten werden uitgevoerd bij proefdieren, vrijwilligers en patiënten.

In *hoofdstuk 2* wordt de ademrespons op hypercapnie (verhoogde arteriële koolzuurspanning) en hypoxie (verlaagde arteriële zuurstofspanning) beschreven bij gezonde vrijwilligers en patiënten bij wie het glomuslichaampje (*glomus caroticum, carotid body*), enkel- of dubbelzijdig, operatief is verwijderd vanwege een glomustumor. In het *glomus caroticum* bevinden zich de perifere chemoreceptoren. De ventilatoire respons op een stapsgewijze verhoging van de eind-expiratoire koolzuurspanning ($P_{ET}CO_2$) is te verdelen in een snelle ($\tau \sim 10$ s) en een langzame ($\tau \sim 120$ s) component. Aangenomen wordt dat de snelle component z'n origine heeft in de perifere chemoreflexus (met als sensoren de perifere chemoreceptoren in het *glomus caroticum*), terwijl de langzame component afkomstig is van de centrale chemoreflexus (met als sensoren de centrale chemoreceptoren in de ventrale medulla). Deze studie is een poging om bij de mens de hypothese te valideren dat het *glomus caroticum* de sensoren bevat waaruit de snelle ventilatoire component voortkomt (tijdens stimulatie met koolzuur). De resultaten laten zien dat bij personen, die een dubbelzijdige *glomus caroticum* resectie hebben ondergaan de dynamische \dot{V}_T-CO_2 respons het best te beschrijven is met één (langzame) component en dat er geen verbetering optreedt na toevoeging van een tweede component. Doet zich een verbetering voor met de introductie van een tweede (snelle) component, dan wordt dit geassocieerd met de aanwezigheid van een perifere chemoreflex respons. Dit was alleen het geval bij de groep éézijdig geopereerde patiënten en bij de

controle groep. Onze resultaten valideren de hypothese dat de snelle component van de ventilatoire respons op hypercapnie afkomstig is van de perifere chemoreceptoren in het glomus caroticum.

Het effect van een antioxidantcombinatie (vitamine C en E) op de door halothaan geïnduceerde depressie van de ventilatoire acute hypoxische respons (AHR) wordt in *hoofdstuk 3* beschreven. Halothaan veroorzaakt, reeds in zeer lage concentraties (0.05–0.1 eind-expiratoir (ET) %), een uitgesproken depressie van het glomus caroticum en daardoor van de AHR. Het mechanisme dat verantwoordelijk is voor 'O₂-sensing' in het glomus caroticum (en dus voor de AHR) is niet bekend, maar de literatuur geeft aan dat de betrokkenheid van vrije radicalen goed mogelijk is. Van halothaan is het bekend dat het onder hypoxische omstandigheden vrije radicalen produceert. De resultaten tonen aan dat een halothaan concentratie van 0.11 ET % de AHR deprimeert met meer dan 50%. Deze depressie werd volledig voorkomen door een voorbehandeling met de vitaminedcocktail, maar niet door voorbehandeling met placebo. Deze opmerkelijke bevinding vergoot ons inzicht niet alleen in het mechanisme van 'O₂-sensing', maar ook in het mechanisme van halothaan-geïnduceerde ademdepressie. Tenslotte bieden deze bevindingen nieuwe mogelijkheden voor therapeutisch ingrijpen om potentiële levensbedreigende situaties in de postoperatieve periode, na gebruik van inhalatie anesthetica, te voorkomen.

Onderzoek naar de effecten van het populaire intraveneuze anestheticum propofol op het ademregelsysteem wordt beschreven in de *hoofdstukken 4 en 5*. Het effect van propofol op tal van cardio-respiratoire parameters wordt bestudeerd tijdens lucht ademen (rust \dot{V}_i), hypercapnische stimulatie, kort- en langdurige hypoxie bij gezonde vrijwilligers.

In *hoofdstuk 4* wordt aangetoond dat propofol (in een zeer lage plasmaconcentratie van circa 0.6 $\mu\text{g/ml}$) de acute hypoxische respons deprimeert met ~50%. Een interessante bevinding is de afwezigheid van de ontwikkeling van secundaire depressie van de ventilatie door hypoxie (hypoxische ventilatoire depressie of HVD) gedurende 5 opeenvolgende korte (3 min) perioden van hypoxie, terwijl eenzelfde periode aaneengesloten hypoxie (15 min) wel HVD doet ontstaan. Dit fenomeen is propofol-onafhankelijk. Omdat in dit protocol de $\dot{V}_i\text{-CO}_2$ onbestudeerd bleef, is het aangrijpingspunt van propofol binnen het ademregelsysteem niet duidelijk geworden. De door propofol veroorzaakte ademhalingsdepressie kan tot stand zijn gekomen door een effect op de perifere of centrale chemoreflexus, of secundair zijn aan de door propofol veroorzaakte sedatie.

De lokatie van het aangrijpingspunt van propofol binnen het ademregelsysteem wordt verduidelijkt in *hoofdstuk 5*. Het effect van propofol op de dynamische $\dot{V}_i\text{-CO}_2$ respons werd gemeten. Sedatieve propofolconcentraties veroorzaken een exclusieve depressie van de centrale chemoreflexus (de perifere chemoreflexus blijft onaangestast door propofol). Dit is een bijzondere bevinding die in contrast staat met het feit dat inhalatieanesthetica een selectieve depressie geven van de perifere chemoreflexus. We schrijven dit toe aan het verschil in het werkingsmechanisme van propofol en inhala-

tieanesthetica. Het is zeer goed mogelijk dat het waargenomen verschil gerelateerd is aan de antioxidatieve werking van propofol tegenover de pro-oxidatieve werking van inhalatieanesthetica (zie *hoofdstuk 3*).

In de *hoofdstukken 6 en 7* wordt het effect van de gecombineerde toediening van opioïden en anesthetica onderzocht. Dit gebeurt met behulp van de techniek van responsoppervlak-analyse. Deze techniek stelt ons in staat om 3D representaties van de concentratie-respons relatie te construeren alsmede de wijze van interactie (additief, synergistisch of antagonistisch) vast te stellen over het gehele responsoppervlak. Omdat het gecombineerd toedienen van anesthetica en opioïden gemeengoed is in de anesthesiologie, ook in spontaan ademende patiënten, geven deze studies ons informatie die kan leiden tot het maken van doseringsschema's voor optimale analgesie of sedatie met minimale ademdepressie.

Initieel is het effect van het gecombineerd toedienen van een opioïd, alfentanil, en een inhalatieanestheticum, sevofluraan, onderzocht (*hoofdstuk 6*). De invloed van verschillende concentraties van alfentanil alleen (0-50 ng/ml) en sevofluraan alleen (0-0.4 ET%) en meerdere concentratiecombinaties werd gemeten op CO_2 -gestimuleerde ventilatie en de acute hypoxische respons. Alfentanil en sevofluraan hebben een dosis afhankelijk deprimerend effect op beide parameters. In combinatie was het effect op de CO_2 -gestimuleerde ventilatie synergistisch, op de AHR additief. De depressie van de AHR treedt op bij veel lagere concentraties van beide middelen (zowel indien separaat toegediend als in de combinatie).

Vervolgens werd de interactie van een intraveneuze anestheticum, propofol, en een opioïd, remifentanil, bestudeerd (*hoofdstuk 7*). Remifentanil en propofol gaven een dosis afhankelijke respiratoire depressie. Tijdens de gecombineerde toediening is het deprimerende effect uitgesproken synergistisch. De resultaten duiden er verder op dat indien de anesthesioloog tijdens de anesthesie met de combinatie remifentanil/propofol meer of minder sedatie wenst hij of zij er verstandig aan doet de propofolconcentratie te wijzigen. Dit zal weinig tot geen additioneel effect op de ademhaling hebben. Is echter toch minder ademdepressie gewenst dan heeft het slechts zin de remifentanil concentratie te wijzigen. Dit zal weinig effect hebben op het sedatie/hypnose niveau van de patient.

In *hoofdstuk 8* wordt het effect van tramadol op het ademregelysteem onderzocht in een experimenteel kat model. Tramadol is een analgeticum met zowel opioïde als non-opioïde werkingsmechanismen. Het resultaat van deze studie is dat minstens 70% van het ademdeprimerende effect van tramadol tot stand komt via opioïd-receptoren.

De ademregulatie in de postoperatieve fase werd gemeten in patiënten na uitgebreide abdominale chirurgie en twee tot drie dagen intensieve zorg (*hoofdstuk 9*). Het doel van de studie is te bestuderen of deze patiënten in staat zijn om adequaat te reageren op perioden van bovenste luchtwegobstructie. Veranderingen in de inspiratoire gasconcentraties werden toegediend alsware het veranderingen in de chemische samenstelling

van het bloed tijdens een bovenste luchtwegobstructie. Dit resulteerde in een geleidelijke toename in de $P_{ET}CO_2$ al dan niet gecombineerd met een geleidelijke daling van de arteriële zuurstofsaturatie. De ventilatiereactie werd vervolgens gemeten. Patiënten werden zowel direct postoperatief als 6 tot 8 weken later onderzocht. De resultaten laten zien dat initieel maar niet tijdens de vervolgstudie, alle patiënten een verhoogde bloedspiegel van het C-reactief eiwit hadden (een teken van een actief onstekingsproces). Terwijl de morfine plasmaconcentratie relatief hoog was bleek de respiratoire depressie bescheiden te zijn in de postoperatieve fase. Dit staat in schril contrast tot de bevinding bij gezonde proefpersonen die ernstige ademdepressie tonen na toediening van morfine (leidend tot plasmaconcentraties zoals bij onze patiënten gemeten). Dit is niet verbazingwekkend als men in ogenschouw neemt, dat de ademhaling bij patiënten bepaald wordt door de balans tussen stimulatie door pijn en stress, het ontstekingsproces en geactiveerde chemoreflexen enerzijds en depressie door sedatie en de direct deprimerende effecten van anesthetica en analgetica op respiratoire neuronen in het perifere en centrale zenuwstelsel anderzijds. Onze data suggereren dat de ademrespons, die nodig is om hypoxie en hypercapnie te herstellen na of tijdens een bovenste luchtwegobstructie redelijk intact is.

IN CONCLUSIE, de data beschreven in dit proefschrift tonen het volgende aan:

- De snelle ventilatoire component tijdens koolzuurstimulatie is afkomstig van de perifere chemoreceptoren in het glomus caroticum;
- De depressie van de hypoxische ventilatoire respons door lage concentraties halothaan is te voorkomen door voorbehandeling met antioxidanten;
- Een lage dosis propofol deprimeert de acute hypoxische ventilatoire respons met 50%;
- Propofol deprimeert de dynamische \dot{V}_T - CO_2 respons *via* een exclusief effect binnen de centrale chemoreflexus;
- Het combineren van lage doses opioïden en anesthetica leidt veelal tot synergistische interacties met forse ademdepressie die het best wordt tegengegaan door de opioïdconcentratie te verlagen;
- Ademdepressie door tramadol wordt voor minstens 70% bepaald door interactie met opioïdreceptoren;
- Bij patiënten na een grote buikoperatie en intensieve zorg lijkt de ademhalingsdepressie bescheiden te zijn.

In de studies beschreven in dit proefschrift is waardevolle informatie over het (cardio)respiratoir regelsysteem bij de perioperatieve patiënt verkregen. Het preciese mechanisme en het complex aan factoren dat een rol speelt bij de perioperatieve veranderingen in het ademregelsysteem blijft grotendeels onduidelijk. Opheldering hiervan is

van grote waarde voor de klinische praktijk. Toekomstige studies zullen zich moeten richten op de interactie van de chirurgische stress, postoperative pijn en pijnstilling, bovenste luchtwegobstructie, zuurstoftherapie en de effecten van anesthetica en analgetica op dit belangrijke regelsysteem. Een eerste poging door ons ondernomen wordt beschreven in *hoofdstuk 9*. In deze studie vonden we een bescheiden effect op de ademhaling na uitgebreide abdominale chirurgie, ondanks relatief hoge morfineconcentraties. Dit suggereert dat de ventilatoire respons, die nodig is om de bloedgaswaarden te herstellen na of tijdens een bovenste luchtwegobstructie, redelijk efficiënt zou functioneren in de postoperatieve patiënt. Echter het ruime aantal studies in de literatuur, dat laat zien dat bij postoperatieve patiënten frequente perioden van soms diepe hypoxie voorkomen, suggereert het tegenovergestelde. Verder onderzoek zal nodig zijn om ons inzicht in deze materie te vergroten.

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List of Abbreviations and Symbols

<i>Main Respiratory Terms</i>		<i>Modifiers</i>	
AHR	acute hypoxic response	<i>a</i>	arterial
B_k	apneic threshold	<i>C</i>	central loop related
CO_2	carbon dioxide	<i>e</i>	expired
<i>F</i>	fraction	<i>ET</i>	end-tidal
<i>G</i>	chemoreflex gain	<i>i</i>	inspired
HVD	hypoxic ventilatory decline	<i>n</i>	parallel noise
O_2	oxygen	<i>P</i>	peripheral loop related
<i>P</i>	partial pressure	<i>T</i>	total
<i>RR</i>	respiratory rate		
<i>S</i>	ventilatory CO_2 sensitivity		
S_pO_2	oxygen saturation		
<i>T</i>	time delay		
τ	time constant		
\dot{V}_i	inspired minute ventilation		
V_T	tidal volume		
<i>W</i>	measurement noise		

Modeling Terms of Chapters 6 and 7

AIC	Akaike's information criterion
C_{25}	concentration causing 25% effect
C_{50}	concentration causing 50% effect
<i>E</i>	effect
E_0	baseline effect
γ	shape parameter
λ	scaling parameter

Miscellaneous Terms

AOX	antioxidant
BIS	bispectral index
CBF	cerebral blood flow
CBR	carotid body resection
DEF	dynamic end-tidal forcing
EEG	electroencephalogram
GABA	γ amino butyric acid
HR	heart rate
MAP	mean arterial pressure
MFBS	multifrequency binary sequence
OR	opioid receptor
ROS	reactive oxygen species

Curriculum Vitae

Diederik Jan Friso Nieuwenhuijs was born on Februari 8, 1972 in Utrecht, The Netherlands. He obtained his Atheneum diploma at the Sint Bonifatius college in Utrecht in July 1990. In September 1990 he entered medical school at the Vrije Universiteit in Amsterdam and received his medical degree in June 1998. From June to October 1998 he worked as a resident at the Intensive Care Unit at the Elisabeth Gasthuis in Eindhoven. In October 1998, he was appointed as graduate student, supported by Grant MW 902-19-144 from The Netherlands Organization for Pure Research (ZorgOnderzoek Nederland Medische Wetenschappen-NWO), at the Department of Anesthesiology, Leiden University Medical Center, and started the investigations described in this thesis. In October 2002, he will start his residency in anesthesiology at the Department of Anesthesiology at the Leiden University Medical Center (Chairman: Prof. Dr. J.W. van Kleef).

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Stellingen behorende bij het proefschrift

CardioRespiratory Control in the Perioperative Patient: *from bench to bedside*

1. Ademhalingsdepressie, veroorzaakt door het effect van subanesthetische concentraties van inhalatieanesthetica op de perifere chemoreceptoren (gelocaliseerd in het glomus caroticum), is effectief te antagoneren door de toediening van antioxidanten.
Hoofdstuk 3 van dit proefschrift.
2. In tegenstelling tot de inhalatieanesthetica heeft propofol geen selectief ademhalingsdeprimerend effect op de perifere chemoreceptoren. Dit is hoogstwaarschijnlijk gerelateerd aan de antioxidatieve eigenschappen van propofol.
Hoofdstuk 5 van dit proefschrift.
3. De ademhaling bij patiënten, na een grote buikoperatie en postoperatieve pijnstilling door morfine, is gedeprimeerd, ondanks stimulerende invloeden van pijn en stress.
Hoofdstuk 9 van dit proefschrift.
4. De bispectrale index van het EEG is niet in staat de sedatieve effecten van opioïden aan te tonen, zelfs niet indien deze worden gecombineerd met een anestheticum.
Hoofdstuk 6 en 7 van dit proefschrift.
5. In tegenstelling tot morfine heeft morfine-6-glucuronide nauwelijks effect op de isocapnische hypoxische ventilatoire respons. Dit is een belangrijke aanwijzing dat deze opioïden hun werking hebben via een separaat receptorsysteem.
6. Ondanks het gunstige bijwerkingsprofiel van morfine-6-glucuronide zal het alleen dan een acceptabel alternatief voor morfine zijn indien de gebruiksprijs vergelijkbaar is aan die van morfine.
7. De 'bootstrap' techniek is geschikt om het meest adequate model uit een serie niet geneste modellen te selekteren.
8. De bloed-biofase equilibratie constante (k_{eo}) is groter voor de effecten op de ademhaling dan voor de analgetische effecten van intraveneus toegediende opioïden. Dit kan duiden op een verzadigingsfenomeen in de banen die betrokken zijn bij de ademhalingseffecten.

9. Ontdekken is zien wat iedereen heeft gezien en denken wat niemand heeft gedacht.
Albert Szent-Györgyi.
10. Budgettering van de gezondheidszorg en het wegwerken van wachtlijsten gaan niet samen.
11. Vernieuwing in de politiek : de "sorry" cultuur van Paars is veranderd in de "zo had Pim het gewild" cultuur van LPF.
12. Om iemands wind uit de zeilen te nemen, moet het diegene wel voor de wind gaan.

Diederik Nieuwenhuijs

Leiden, 4 september 2002

