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 (τ ~10 s) and a slow component τ ~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 oxireductase 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 $ASP^{92} \Rightarrow$ 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 ⊤-piece. One arm of the ⊤-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 O_2, N_2 and CO_2 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 O_2 and CO_2 concentrations and the arterial hemoglobin-O_2 saturation (S_PO_2) were measured with a Datex Multicap gas monitor (near the mouth) and Datex Satelite 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 PCO_2 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 CO_2: 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 CO_2 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 CO_2 (*cf. Chapter 4*).

*See for the rationale of using MFBS rather than step CO_2 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_PO_2 ~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:

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

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

$\dot{V}_c(t)$ 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]

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

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:

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

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_PO_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]

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

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 ± SD. P-values $< 0{\cdot}05$ 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 ($P < 0{\cdot}05$). All other parameters were unaffected by hypoxia, with the exception of G_C in control subjects which increased significantly ($P < 0{\cdot}05$).

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 min^{-1} kPa^{-1}	L min^{-1} kPa^{-1}	kPa	s	s	s	s	ml min^{-2}
—Two-Compartment Model: Bilateral Carotid Body Resection—								
normoxia	7·1 ± 5·3	1·6 ± 1·2	4·5 ± 0·5	116 ± 71	11 ± 11	13 ± 8	9 ± 4	80 ± 40
hypoxia	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—								
normoxia	10·4 ± 3·3	2·2 ± 1·4	4·6 ± 0·4	103 ± 87	3 ± 6	9 ± 5	7 ± 2	120 ± 90
hypoxia	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—								
normoxia	10·3 ± 4·2	3·1 ± 2·2	4·9 ± 0·3	148 ± 69	6 ± 7	12 ± 5	6 ± 1	60 ± 60
hypoxia	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—								
normoxia	7·9 ± 4·6	–	4·4 ± 0·5	87 ± 37	–	6 ± 2	–	90 ± 50
hypoxia	7·9 ± 2·5	–	4·5 ± 0·3	92 ± 87	–	7 ± 4	–	60 ± 70

Values are mean ± 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.

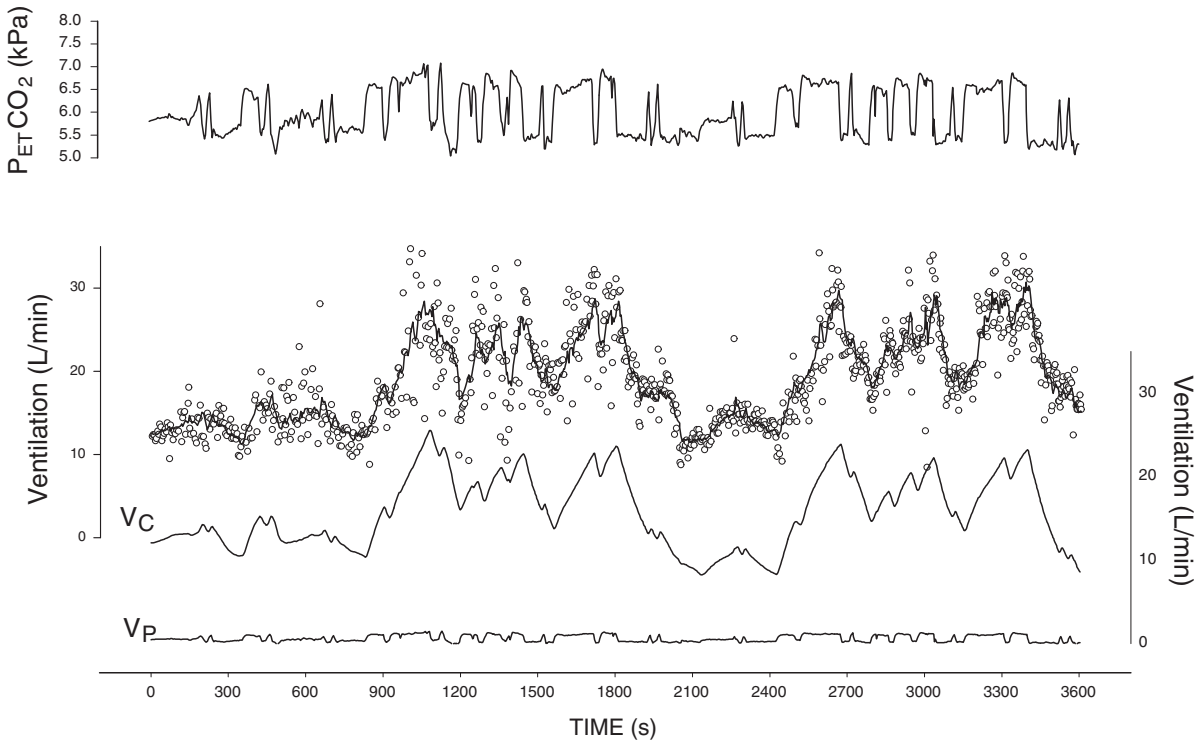

Figure 1. Two-compartment model fit to the normoxic CO_2 data of a bilaterally CBR patient. Shown is the response to 2 subsequent MFBS CO_2 inputs. Each dot is one breath. The line through the data points is the sum of the peripheral component ($\dot{V}_P$), central component ($\dot{V}_C$), parallel 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 ($P < 0{\cdot}05$). 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 ($P < 0{\cdot}05$). 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: $0{\cdot}12 \pm 0{\cdot}09$, $0{\cdot}53 \pm 0{\cdot}43$ ($P = 0{\cdot}03$ *vs.* bilateral CBR), and $1{\cdot}33 \pm 0{\cdot}80$ L min^{-1} %$^{-1}$ ($P = 0{\cdot}03$ *vs.* unilateral CBR and $P < 0{\cdot}01$ *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 $0{\cdot}35 \pm 0{\cdot}27$, unilateral CBR $0{\cdot}46 \pm 0{\cdot}34$ and control $0{\cdot}83 \pm 0{\cdot}65$ L min^{-1} %$^{-1}$ ($P = 0{\cdot}11$).

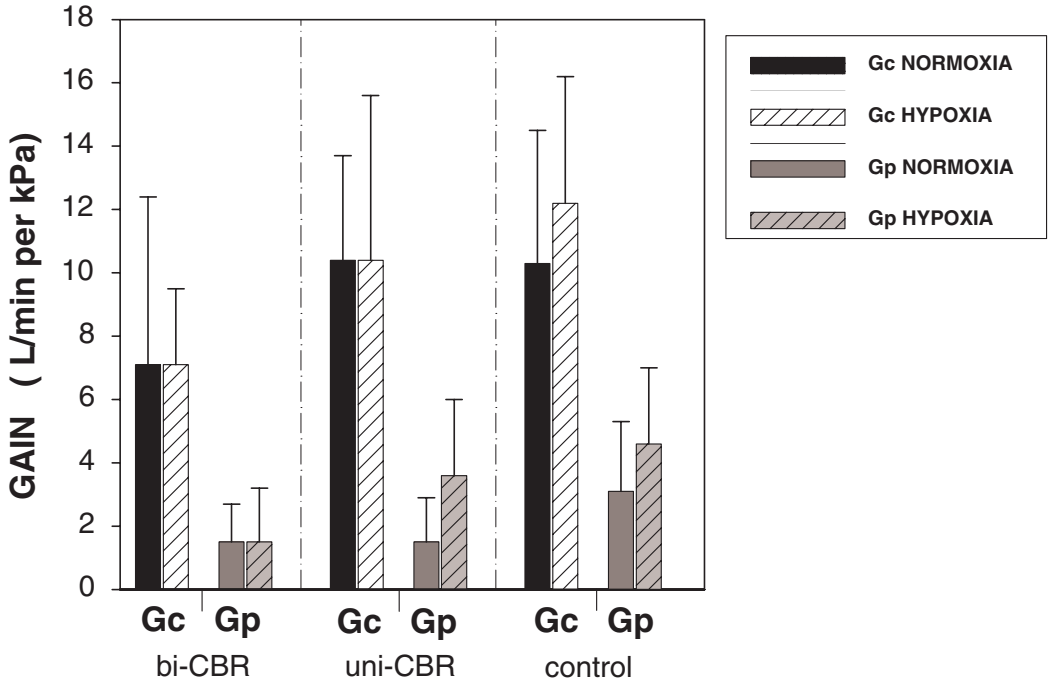

Figure 2. Mean values ± SD of the gain's of the two-compartment model for bilateral and unilateral CBR patients and control subjects. See text for the result of the paired (effect of hypoxia) and unpaired comparisons (differences among the three groups).

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

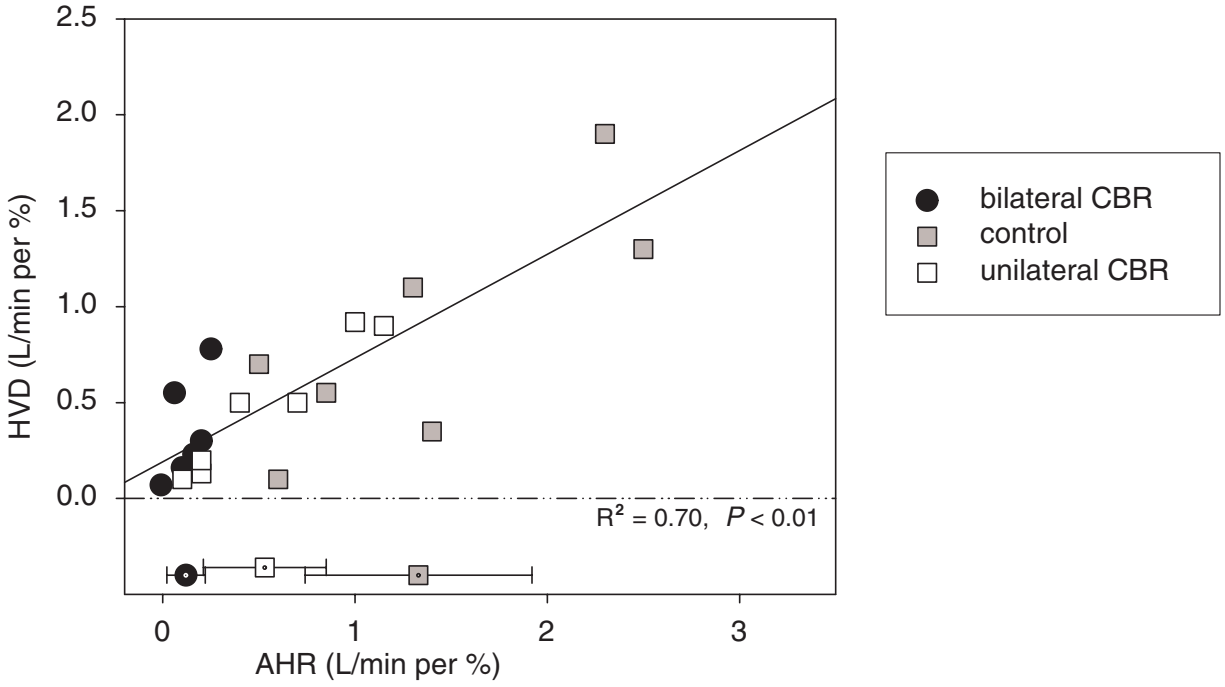

Figure 3. The acute hypoxic response (AHR) *versus* the hypoxic ventilatory decline (HVD). The continuous line is the linear regression. On the bottom the mean ± 95% confidence interval AHR-values for the three groups is given.

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*