

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).

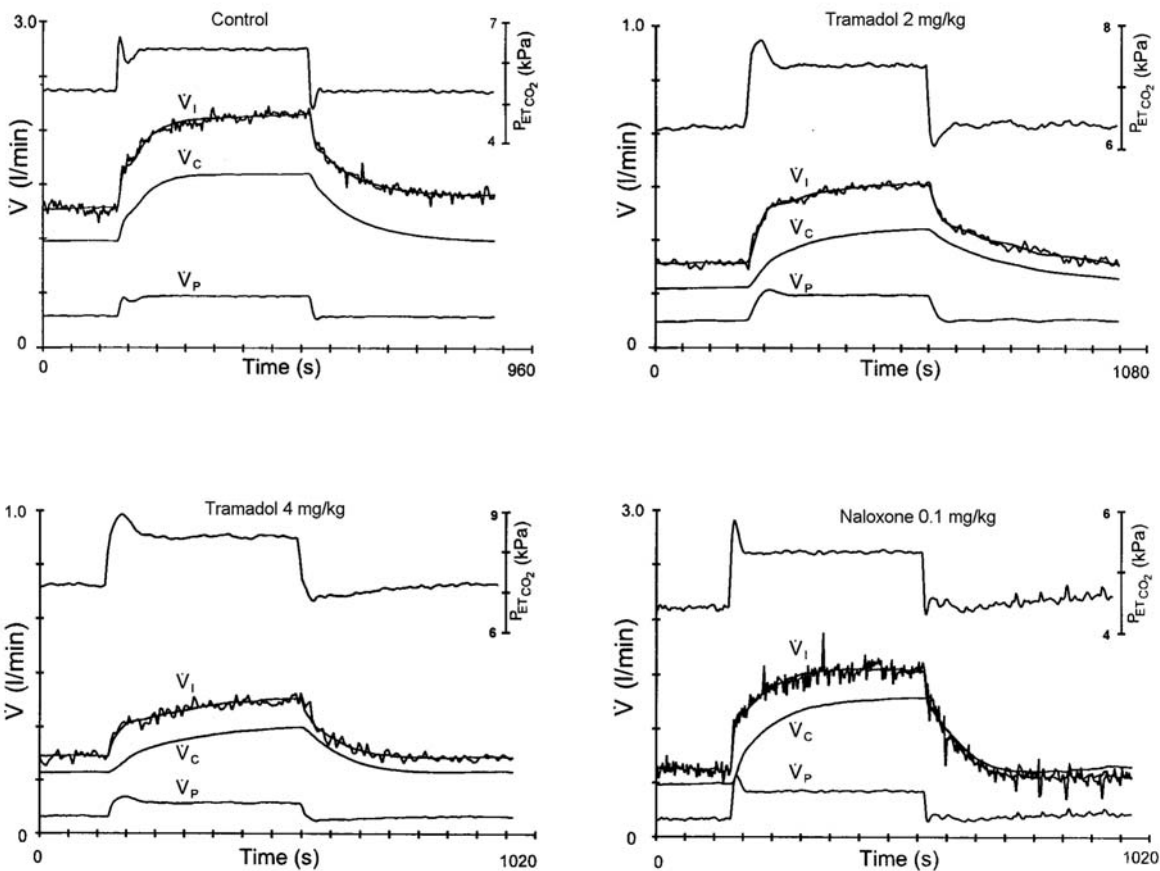


Figure 1. Examples of the DEF runs in one animal from group 1 showing the effects of control, 2 and 4 mg/kg tramadol, and (last panel) the total reversal of tramadol effect after naloxone (0.1 mg/kg) infusion. The top diagram in each panel is the P_{ETCO_2} input function. The line through the \dot{V}_i 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-

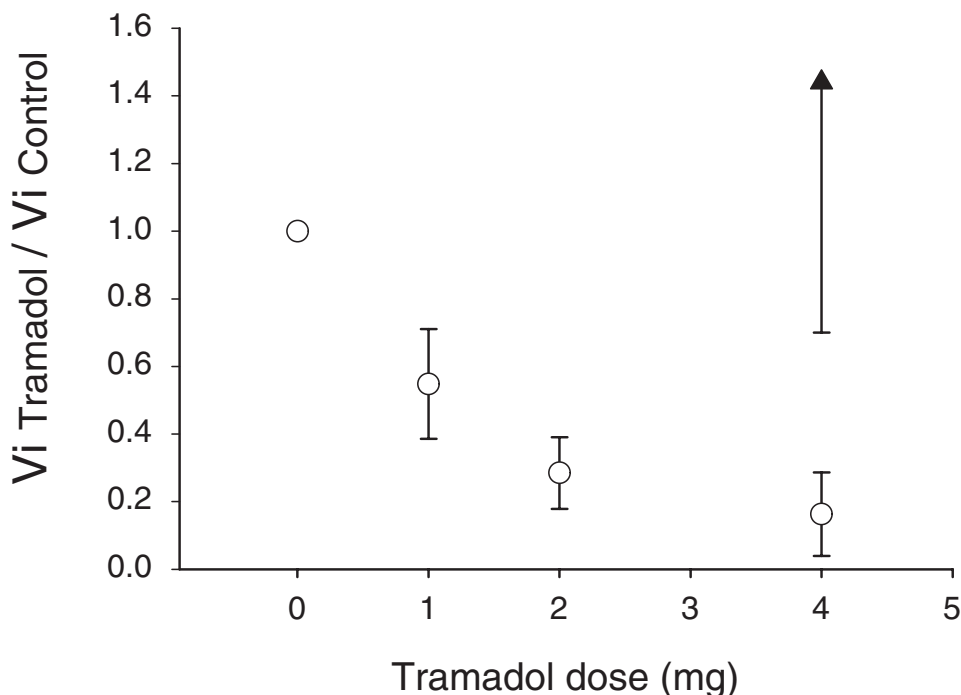


Figure 2. The dose-dependent decrease in relative ventilation at a fixed $P_{ET}CO_2$ of 6 kPa (\circ) in 5 animals. The triangle depicts the effect of naloxone given after 4 mg/kg tramadol.

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-

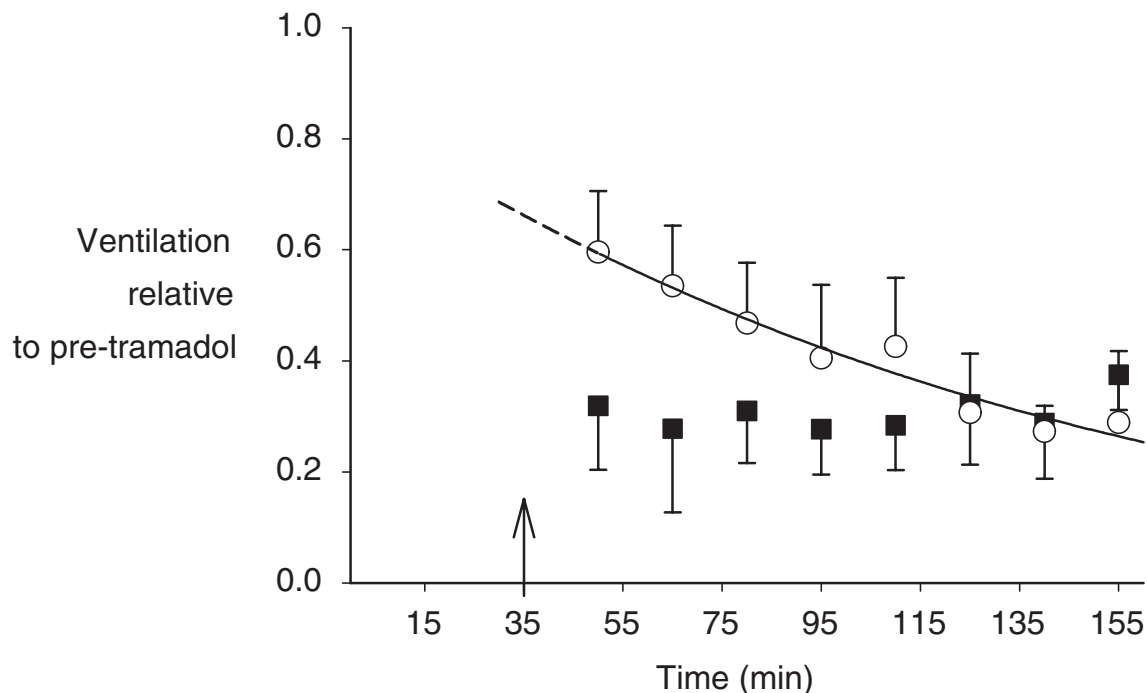


Figure 3. Open symbols: Influence of naloxone pretreatment (0.1 mg/kg, given at time=0) on ventilation at a fixed $P_{ET}CO_2$ of 6 kPa after infusion of 4 mg/kg tramadol (arrow) in five animals. Closed symbols: The same single dose of tramadol in five animals not pretreated with naloxone. The continuous 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

