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The effect of almitrine bismesylate on the steady-state responses of arterial chemoreceptors to CO_2 and O_2 in the cat

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Abstract. The interaction between almitrine bismesylate, a pharmacological stimulant of peripheral chemoreceptors, and varying levels of oxygen (P_{O_2} 50–600 Torr) and carbon dioxide (P_{CO_2} 10–65 Torr) on steady state carotid chemoreceptor discharge was investigated in pentobarbitone-anaesthetised cats. Almitrine was given as constant intravenous (50 μg/kg per min for 4 min) and intracarotid infusions (4–16 μg/kg per min) at different levels of alveolar P_{O_2} and P_{CO_2}. Almitrine always excited discharge. The intracarotid infusions at the lower infusion rate (4–8 μg/kg per min) and the i.v. infusions increased the slope of the isoxic response to CO_2. This effect could be reversed by raising P_{O_2} to high levels. Higher infusion rates of almitrine (16 μg/kg per min) displaced the CO_2 response curve upwards but did not increase its slope above that obtained in control conditions at end-tidal P_{O_2} of 50 Torr. However, as these higher infusion rates caused levels of discharge greater than those achieved during control conditions, their effects on control CO_2 sensitivity could not be ascertained. Our results suggest that almitrine excites carotid body chemoreceptors by a mechanism similar to that of hypoxia and not like that of carbon dioxide.

Almitrine; Carotid body chemoreceptor; Hypercapnia; Hypoxia

Almitrine bismesylate is a powerful ventilatory stimulant with a specific action on peripheral chemoreceptors (Laubie and Diot, 1972; Laubie and Schmitt, 1980). In normal man almitrine augments the ventilatory response to hypoxia (Stradling *et al.*, 1982), but there is some disagreement about its effect on ventilatory sensitivity to CO_2 (Stradling *et al.*, 1982; Stanley *et al.*, 1983). We have therefore studied the steady-state interactions between the effects of almitrine and those of the natural stimuli of the carotid body (hypoxia and hypercapnia) on the discharge of carotid body chemoreceptors with a view to determining whether the drug acts in a way similar to hypoxia, hypercapnia, both (asphyxia) or neither. Preliminary accounts of these findings have already been given (Hughes *et al.*, 1986, 1987).

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Methods

Experiments were performed on 13 young cats of either sex (body weight 2.0–2.6 kg), anaesthetised with pentobarbitone (42 mg/kg i.p., supplemented i.v. as required). One femoral artery and both femoral veins were cannulated, the former for withdrawal of 0.6 ml blood samples for blood gas analysis, the latter for infusion of drugs. The left carotid sinus nerve was exposed and the discharge of strands containing only one or a few active chemoreceptor fibres was recorded using the lateral approach of Goodman (1973). The chest was opened widely along the midline so that the animal's own ventilatory efforts could not affect the composition of lung gases. The animals were then ventilated at 2 Hz at a tidal volume of 40 ml with inspired gas concentrations under the control of a microcomputer (Research Machines 380Z) as described by Kumar *et al.* (1988).

The ventilating system allowed us to establish each steady-state $P_{ET_{CO_2}}/P_{ET_{O_2}}$ combination within about 15 sec. Tracheal gas concentrations were continuously sampled by mass spectrometer (VG Micromass model 201). With this technique arterial blood gas changes correspond well with end-tidal gas tensions and the relationship between end-tidal gas tension and the discharge of chemoreceptors is stable over the course of several experimental runs (Kumar *et al.*, 1988).

Rectal temperature was continuously monitored and maintained at 37.5 °C by heating elements under the copper surface of the operating table. In 5 cats the carotid artery was cannulated retrogradely via the posterior auricular artery, the tip of the cannula being positioned just central to the carotid body. This cannula was used for close-arterial infusions of almitrine. At the start of all experiments arterial blood pH was corrected to 7.4 by intravenous infusion of 10% sodium bicarbonate, diluted 1 : 1 with normal saline.

PROTOCOLS

Changes in inspired gases. To measure carotid chemoreceptor activity at different combinations of pulmonary gas tension $P_{ET_{CO_2}}$ was usually held at one of three or four different levels (10–12, 20, 35, and 50 Torr) and $P_{ET_{O_2}}$ was cycled sequentially between approximately 125, 75, 50 and 90 Torr for periods of 40–60 sec each. For any steady $P_{ET_{CO_2}}$ at least five $P_{ET_{O_2}}$ changes were made *i.e.* more than one complete cycle, until chemoreceptor discharge was seen to be stable and reproducible. A new $P_{ET_{CO_2}}$ was then established and the sequence continued. In this way 12–16 discrete points were obtained at different values of $P_{ET_{CO_2}}$ and $P_{ET_{O_2}}$ within about 15 min. In four cats the range of inspired $P_{ET_{O_2}}$ was increased up to 600 mm Hg and $P_{ET_{CO_2}}$ extended to 70 mm Hg. Although the sequence of $P_{ET_{O_2}}$ changes was fixed for any set of measurements, $P_{ET_{CO_2}}$ was not changed at any particular level of $P_{ET_{O_2}}$ and was not altered in any systematic order. In four cats $P_{ET_{O_2}}$ was held constant at each level while $P_{ET_{CO_2}}$ was cycled.

Almitrine infusions. Almitrine bismesylate (Servier, France) dissolved in malic acid (15 mg almitrine in 1 ml malic acid) was diluted with 5% dextrose to give a concentration of approximately 850 μg/ml for close arterial infusions and 125 μg/ml for intravenous infusions.

Close arterial almitrine. Before close arterial infusions (7 cats), discharge was recorded during 1 or 2 complete cycles of inspired gas tension. Infusions of almitrine were then given at rates of 4, 8 and 16 μg/kg per min for 15–20 min each at flow rates of 0.01 to 0.05 ml/min. After any change in infusion rate at least 4 min elapsed before data was collected.

Intravenous almitrine. In four cats almitrine was given as short intravenous infusions (50 μg/kg per min for 4 min). Whilst nerve discharge increased (in response to the almitrine), $P_{ET_{CO_2}}$ was held constant and $P_{ET_{O_2}}$ was cycled. Following this short infusion, normoxic discharge reached the intensity previously given by $P_{ET_{CO_2}}$ and $P_{ET_{O_2}}$ together at 50 Torr each; the discharge declined very little during the next 10 to 15 min so that the response to combinations of hypoxia and hypercapnia were observed at a reasonably constant level of stimulation.

Control infusions. In 4 cats close arterial infusions of 5% dextrose and of malic acid were given at rates equivalent to the almitrine infusion rate of 16 μg/kg per min.

Data collection. End-tidal P_{O_2} and P_{CO_2}, and arterial blood pressure were continuously recorded on both a penwriter (Grass Instruments model 7D) and a magnetic tape recorder (Racal Store 7). Discharge was displayed on an oscilloscope and passed through a window discriminator to an electronic counter. Discharge frequencies were displayed as 5 or 10 sec ramps (height proportional to frequency) on the penwriter and these were used as a check on the stability of the preparation and, when necessary, for the matching of mean discharge frequency to some previously established condition. Nerve discharge was counted and recorded during the final 20 sec of each 40 sec step of the last complete cycle of $P_{ET_{O_2}}$ changes at each level of $P_{ET_{CO_2}}$.

Results

Effects of changing $P_{ET_{O_2}}$ and $P_{ET_{CO_2}}$ on control chemoreceptor discharge. Figure 1 shows a record of $P_{ET_{CO_2}}$, $P_{ET_{O_2}}$ and chemoreceptor discharge during a cycle of $P_{ET_{O_2}}$ changes. This demonstrates the fast response times, stability and repeatability of the cycles of gas tension and chemoreceptor discharge. Approximately 15 min were required to collect data from 12–16 combinations of $P_{ET_{CO_2}}$ and $P_{ET_{O_2}}$.

Effect of almitrine on steady state chemoreceptor discharge. Infusions of the solvent (5% dextrose and malic acid) caused no change in either chemoreceptor discharge or the

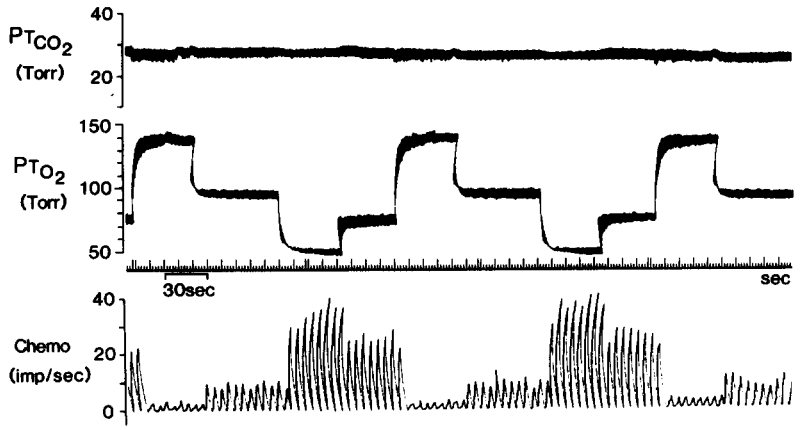

Fig. 1. Chart record of tracheal P_{CO_2} and P_{O_2} and cat carotid chemoreceptor discharge (5 sec ramps) during artificial ventilation at 2 Hz. All P_{O_2} levels were usually held for the same duration – in this example the 100 Torr value is held for longer than the others and shows that steady state discharge is rapidly reached after the change.

response to changes in gas tensions. As previously described (Laubie and Schmitt, 1980) almitrine consistently increased carotid body activity whether given by intravenous or intra-arterial infusion. The effects of the two routes of infusion were similar except that lower intra-arterial infusion rates were needed to cause a given degree of chemoreceptor stimulation.

Increased chemoreceptor discharge became evident within 2 min of an increase in the close-arterial infusion rate of almitrine and within 3 min of an intravenous infusion. Discharge then increased over the course of 4–5 min before levelling off. In these preparations discharge rates at the beginning of a single cycle of $P_{ET_{O_2}}$ changes were within 10% of those at the end, a period of approximately 3 min. With several cycles of inspired gas changes taking about 15 min, discharge rates inevitably increased with the continuing infusion and were less well controlled.

Two patterns of response to close arterial almitrine were observed and related to the total dose of the drug at the time as well as to the infusion rate. At the lower infusion rate of 4–8 μg/kg per min, when the total dose was approximately 100–250 μg/kg, nerve discharge during almitrine infusion did not greatly exceed that of the control data, but the slope of the isoxic CO_2 response was increased at $P_{ET_{O_2}}$ greater than 50 Torr (fig. 2). At infusion rates of 16 μg/kg per min or after total doses greater than 250 μg/kg per min nerve discharge during almitrine infusion became greater than that recorded during control at 50 Torr $P_{ET_{O_2}}$ (fig. 3).

Figure 2 plots typical changes in mean chemoreceptor discharge against $P_{ET_{CO_2}}$ at different $P_{ET_{O_2}}$ during control conditions and during infusions of almitrine at 4–8 μg/kg per min with isoxic points linked together. These lines are taken to represent CO_2 sensitivity of the chemoreceptor fibres at a given P_{O_2}. Nerve discharge did not increase much above that of the control CO_2 sensitivity line at a $P_{ET_{O_2}}$ of 50 Torr.

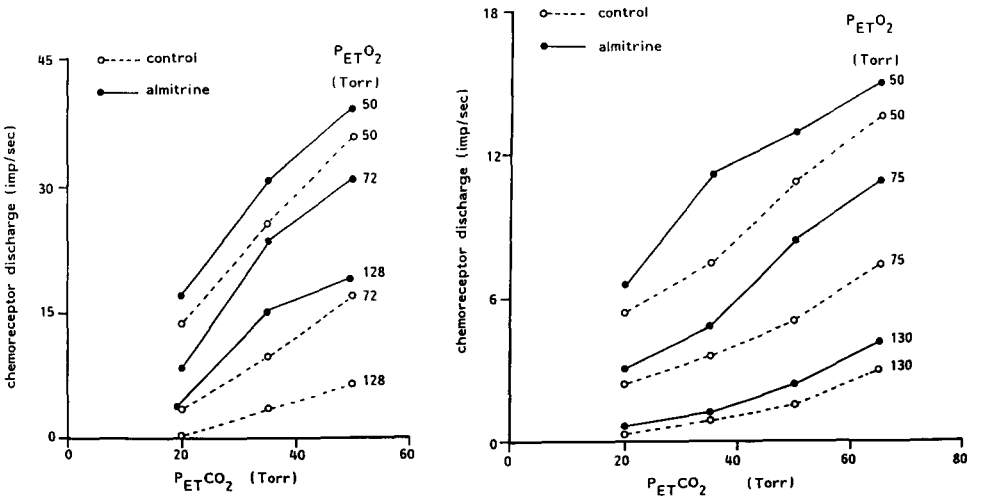

Fig. 2. Two representative plots of carotid chemoreceptor discharge at different PET_{CO_2} and PET_{O_2} during control (——) and during infusion of almitrine at 4–8 μg/kg per min (——). The lines join points of equal PET_{O_2}.

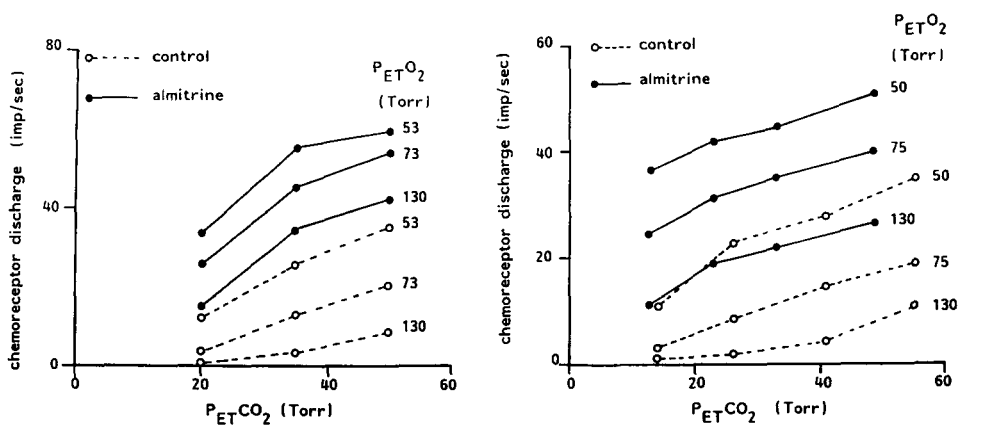

Fig. 3. Two representative plots of carotid chemoreceptor discharge at different PET_{CO_2} and PET_{O_2} during control (——) and during infusion of almitrine at 16 μg/kg per min or when the total dose of almitrine had exceeded 250 μg/kg (——). The lines join points of equal PET_{O_2}. In these plots almitrine has excited discharge to levels greater than that of the most intense control line. The left hand plot has been previously published in an abstract (Hughes *et al.*, 1986).

Figure 3 plots changes as infusion rates of 16 μg/kg per min or after total doses of greater than 250 μg/kg of almitrine, which had a greater stimulatory effect so that discharge during the infusion was greater than the most hypoxic CO_2 sensitivity line. Gradual increases in chemoreceptor discharge with the continuing infusion may have contributed to irregularity in the isoxic CO_2 sensitivity lines, but the randomised order of changes in PET_{CO_2} will have minimised any systematic change in activity overall both within one preparation and between preparations.

TABLE 1

Individual values and mean (SD) of carotid chemoreceptor CO_2 sensitivities of six cats at 130 Torr $P_{ET_{O_2}}$ expressed as slope (of $P_{ET_{CO_2}}$ against carotid chemoreceptor neural discharge) and intercept on the $P_{ET_{CO_2}}$ axis (for a hypothetical zero neural discharge) during control conditions and during almitrine infusions. The slope is calculated by linear regression of isoxic neural discharge at different $P_{ET_{CO_2}}$ and expressed as percent of control CO_2 sensitivity (at 50 Torr $P_{ET_{O_2}}$). Significant differences between control and almitrine: $^*P < 0.005$; $^{**}P < 0.001$ (paired *t*-test).

Cat	Slope			Intercept (Torr)		
	Control		Almitrine	Control		Almitrine
1	32.2		107.8	19.7		1.3
2	18.7		124.7	19.7		0.0
3	22.1		88.8	22.5		11.6
4	38.6		80.3	9.7		−32.5
5	39.9		82.1	17.5		1.4
6	30.5		91.0	9.6		−7.0
mean	30.3	**	95.8	16.5	*	−4.2
(SD)	(8.6)		(17.2)	(5.5)		(15.0)

Isoxic CO_2 sensitivities in six cats were expressed by calculating the slope of each isoxic line using linear regression. These CO_2 sensitivities were then standardised by expressing them as percentages of the control value at $P_{ET_{O_2}}$ 50 Torr. Table 1 gives the values and mean ($\pm$ SD) for the isoxic slopes and their intercepts (on the $P_{ET_{CO_2}}$ axis) at $P_{ET_{O_2}}$ of 130 Torr during control infusions and during almitrine infusions. CO_2 sensitivity during almitrine infusion at $P_{ET_{O_2}}$ of 130 Torr was significantly greater than the control value at the same $P_{ET_{O_2}}$ ($P < 0.001$; paired *t*-test) confirming that almitrine infusion increased the CO_2 sensitivity of the chemoreceptors. There was no significant difference between the slopes of CO_2 sensitivity lines during control at $P_{ET_{O_2}}$ 50 Torr and those during almitrine infusion at $P_{ET_{O_2}}$ of 130 Torr.

Almitrine in normoxia ($P_{ET_{O_2}}$ of 130 Torr) or moderate hypoxia ($P_{ET_{O_2}}$ of 75 Torr) increased CO_2 sensitivity as seen in the increase in slope of the CO_2 response lines (figs. 2 and 3). However, in more severe hypoxia ($P_{ET_{O_2}}$ of 50 Torr) the CO_2 sensitivity lines were displaced upwards by almitrine rather than steepened (figs. 2 and 3). The high infusion rate of almitrine in normoxia and moderate hypoxia displaced these lines upwards as well as steepened them (fig. 3). However, there were no control data at high discharge rates to compare with the discharge rates obtained with almitrine at $P_{ET_{O_2}}$ of 50 Torr and it was thus not possible to say whether lower levels of P_{O_2} would have further steepened the CO_2 sensitivity lines or behaved as almitrine did in displacing them upwards.

In five cats the isocapnic hypoxic sensitivity of the carotid chemoreceptor during control and during intra-arterial almitrine infusions at 4–8 μg/kg per min was estimated

TABLE 2

Mean (SD) values for isocapnic hypoxic sensitivity of carotid chemoreceptors in five cats during control (C) and during intra-carotid infusion of almitrine (A) at 4–8 μg/kg per min estimated by measuring the increase in discharge frequency on changing from 130 to 75 Torr $P_{ET_{O_2}}$ and from 75 to 50 Torr $P_{ET_{O_2}}$ at 20, 35 and 50 Torr $P_{ET_{CO_2}}$ and expressed as percentages of the control change from 130 to 75 Torr $P_{ET_{O_2}}$ at 35 Torr $P_{ET_{CO_2}}$. Significant differences between control and almitrine: $^*P < 0.05$; $^{**}P < 0.01$ (paired *t*-test).

$P_{ET_{O_2}}$	$P_{ET_{CO_2}}$								
	20 Torr			35 Torr			50 Torr		
	C		A	C		A	C		A
130–75 Torr	44	**	116	100	*	166	158	*	250
	(16)		(42)	–		(62)	(21)		(73)
75–50 Torr	116		123	181		129	247		189
	(59)		(16)	(68)		(58)	(96)		(110)

by measuring the increase in discharge frequency between 130 and 75 Torr $P_{ET_{O_2}}$ and between 75 and 50 Torr $P_{ET_{O_2}}$ at any given $P_{ET_{CO_2}}$. These values were then standardised by expressing them as percentages of the control response between 130 and 75 Torr $P_{ET_{O_2}}$ at 35 Torr $P_{ET_{CO_2}}$ (table 2). Between 130 and 75 Torr $P_{ET_{O_2}}$ almitrine caused significant increases in hypoxic sensitivity. However, this was not the case for changes between 75 and 50 Torr $P_{ET_{O_2}}$. If anything, there was a tendency for a decrease in hypoxic sensitivity at higher values of $P_{ET_{CO_2}}$ (table 2). Similarly, analysis of hypoxic responses with higher infusion rates or higher doses of almitrine showed a tendency for hypoxic sensitivity to diminish; however, interpretation of these data is complicated for the reasons stated above.

Figures 4 and 5 show the effect of short infusions of almitrine (50 μg/kg per min for 4 min) on chemoreceptor discharge. Here control CO_2 sensitivity at different levels of $P_{ET_{O_2}}$ was first established and then almitrine was infused intravenously at 110 Torr $P_{ET_{O_2}}$ and at different $P_{ET_{CO_2}}$ until discharge reached the intensity given by 50 Torr $P_{ET_{O_2}}$ during control conditions. Figure 4 plots CO_2 sensitivity lines during control and those obtained sequentially whilst the almitrine infusion gradually increased chemoreceptor discharge and shows again how CO_2 sensitivity is increased with increasing stimulation from almitrine.

$P_{ET_{O_2}}$ was then increased in stages up to 600 Torr to establish hyperoxic CO_2 sensitivity lines (fig. 5). This figure shows that the increase in CO_2 sensitivity caused by almitrine can be abolished by hyperoxia. During some almitrine infusions the chemoreceptor was silenced by ventilating with 100% O_2 (which decreased $P_{ET_{CO_2}}$ to about 10 Torr), but this was not always the case.

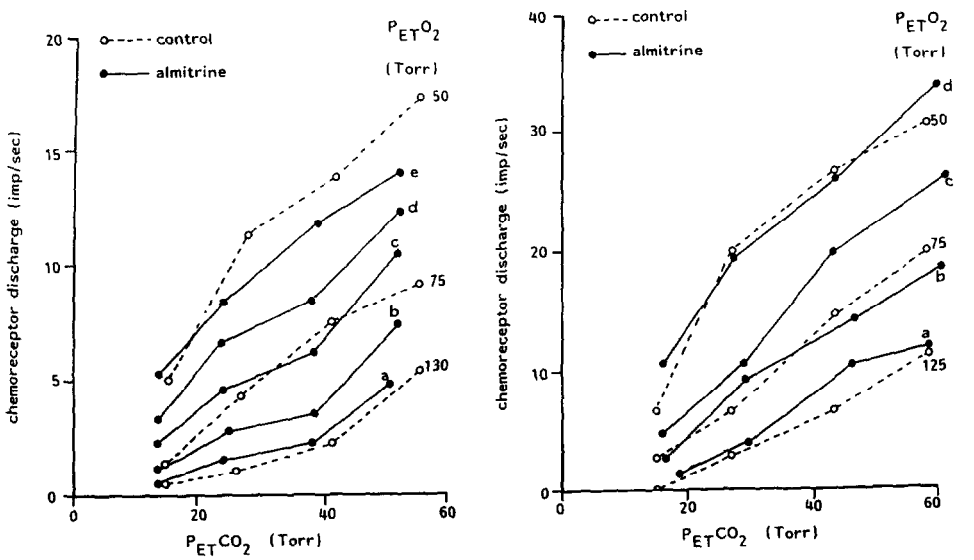

Fig. 4. Representative plots of carotid chemoreceptor discharge *vs* PET_{CO_2} at different PET_{O_2} during control conditions (---) and at a constant PET_{O_2} of 130 Torr during and following 4 min intravenous infusions of almitrine at 50 μg/kg per min (——). The solid lines are numbered alphabetically in the order in which they were obtained and demonstrate the progressive increase in CO_2 sensitivity as almitrine takes effect.

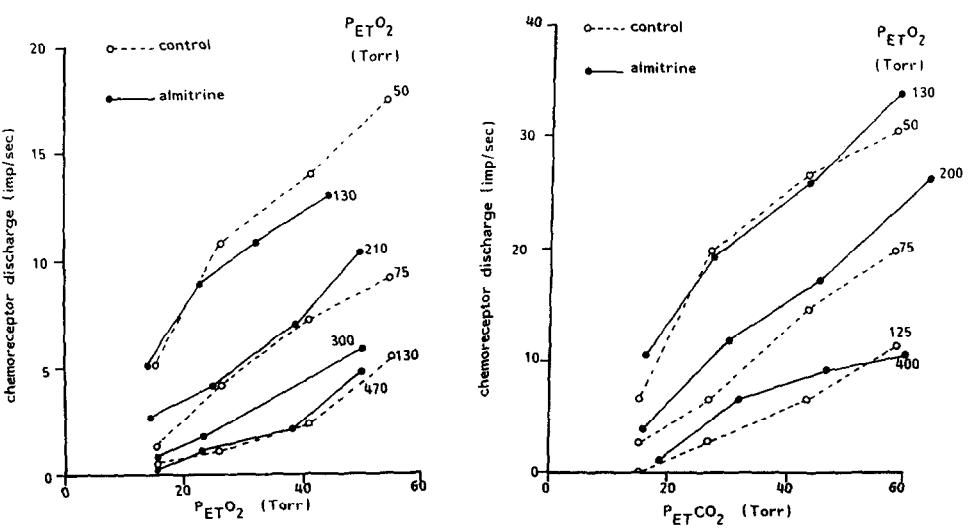

Fig. 5. Representative plots of carotid chemoreceptor discharge *vs* PET_{CO_2} at different PET_{O_2} during control conditions (---) and following 4 min intravenous infusions of almitrine at 50 μg/kg per min, at different PET_{CO_2} and high levels of PET_{O_2} (——). The two preparations shown are those used in fig. 4. Hyperoxia cancels out the response to almitrine.

Discussion

In this study we have confirmed that almitrine excites arterial chemoreceptors. At the lower infusion rates of almitrine (4–8 μg/kg per min) and in normoxia or moderate hypoxia, chemoreceptor sensitivity to changes in $P_{ET_{O_2}}$ and $P_{ET_{CO_2}}$ was also increased. Interpretation of the effects of higher infusion rates was complicated by the lack of control data at equivalent levels of discharge.

Previous studies have reported an increase in hypoxic sensitivity of the carotid body (Bisgard, 1981; Roumy and Leitner, 1981) after almitrine, but only at a single level of P_{CO_2}. There has been no agreement concerning its effects on peripheral CO_2 sensitivity (Bisgard, 1981; O'Regan *et al.*, 1983), although a recent report from Olievier *et al.* (1987) suggests that the ventilatory response to peripheral hypercapnia is increased. With our preparation a large number of measurements at different end-tidal gas concentrations have shown that both CO_2 and hypoxic sensitivity of the carotid body are increased by almitrine.

Almitrine appears to have no direct effect on central respiratory control mechanisms (Laubie and Schmitt, 1980). Indeed the arterial chemoreceptors appear to be its main site of action apart from effects on the pulmonary vasculature, where its action is predominantly independent of the carotid body (Hughes et al., 1983). Its relatively slow onset and prolonged duration of action (in comparison to other chemoreceptor stimulants such as hypoxia or cyanide) has been reported by others (Laubie and Schmitt, 1980; Bisgard, 1981) and are consistent with the idea that the drug is accumulated or retained by the carotid body (Gordon *et al.*, 1987).

The repeatable increase of CO_2 sensitivity when chemoreceptor discharge at any given $P_{ET_{CO_2}}$ did not exceed that obtained at a $P_{ET_{O_2}}$ of 50 Torr (fig. 2) is clearly very similar to the effect of increasing hypoxia on CO_2 sensitivity. This is also seen in the measurements taken in hyperoxia (fig. 5) and suggests that almitrine may act at the same site as hypoxia. The effects on the CO_2 sensitivity lines when these lay above the most hypoxic control slopes (fig. 3) are more difficult to interpret, because at very low $P_{ET_{O_2}}$ all elements of the carotid body may be affected by the severe hypoxia. The finding of an upward shift in the response with, at times, a reduction of the slope suggests that maximal stimulation of the carotid body was being approached.

In conclusion, we have shown that intra-carotid infusion of almitrine, a peripheral chemoreceptor stimulant, increases the sensitivity of arterial chemoreceptors both to hypoxia and to CO_2 in normoxia or moderate hypoxia. The resemblance of this to the effects of hypoxia suggests that both may act by similar or identical mechanisms.

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