The effect of almitrine on the steady-state ventilatory response to carbon dioxide at rest and during exercise in man

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ABSTRACT: Almitrine has potential as a tool for testing the physiological role of the peripheral chemoreceptor. The effects of almitrine on CO_2 chemosensitivity were studied at rest and during light exercise using a constant inflow technique that avoids the hyperoxia of rebreathing methods. The steady-state ventilatory response to CO_2 was measured in two groups of six normal men before and 150 min after 100 mg oral almitrine bismesylate or placebo. One group was studied at rest, the other while pedalling at 50 W. The resting group showed a significant increase in CO_2 response slope after almitrine when compared with placebo but there was no significant change in the response intercept. During exercise the individual results were very variable and after almitrine no significant change was seen in either the response slope or intercept. Control ventilation was not affected by almitrine in either group. Even in the absence of marked hyperoxia the effect of almitrine on CO_2 sensitivity at rest is small. The lack of effect at 50 W is against any important role for the peripheral chemoreceptor during light exercise but other interpretations are possible.

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Almitrine is a piperazine derivative. Because it is insoluble in water, it is used as the bismesylate salt. In animals almitrine increases ventilation, an action which can be blocked by bilateral neurotomy of the carotid sinus and vagal nerves [1]; small doses of almitrine, injected directly into the vertebral artery or intracisternally, have no effect on ventilation [2], suggesting that almitrine stimulates ventilation by an effect on peripheral chemoreceptors. In keeping with this site of action, the effects of almitrine on ventilation are antagonized in part by oxygen and almitrine enhances hypoxic respiratory drive [3, 4].

In the cat OLIEVIER *et al.* [5] have shown that almitrine doubles the peripheral chemoreceptor response to carbon dioxide, *i.e.* in normal man some workers have found a small increase in the ventilatory response to carbon dioxide [6], whilst others have found no significant change [3, 4]. These investigators used the rebreathing method so that the concomitant hyperoxia might have depressed the effects of almitrine. In normal man, there has been only one study of the effect of almitrine on CO_2 responsiveness in normoxia but this showed no effect except at very high doses [7].

We have examined the effect of almitrine on the ventilatory response to CO_2 using a steady-state constant inflow method [8]. As well as avoiding marked hyperoxia, this technique enables the CO_2 response to be studied at physiological levels of carbon dioxide tension (Pco_2) close to the normal control point and has the practical advantage of being more rapid than conventional steady-state methods, requiring, at rest, just 6 min for equilibration. When the method is applied during exercise, only 4 min are required to determine each point and, for small CO_2 loads, the slope of the response obtained tends to increase progressively with the work load [9, 10]. It is not known whether the peripheral chemoreceptor plays any part in this change in CO_2 sensitivity and for this reason the possible effects of almitrine on the exercising CO_2 response are of special interest. Any positive interaction between almitrine and the increased CO_2 sensitivity seen in exercise would be a strong pointer to involvement of the peripheral chemoreceptor.

The resting experiments were reported previously [11] but only five subjects were included as the taped data from a sixth subject were lost and had to be repeated later.
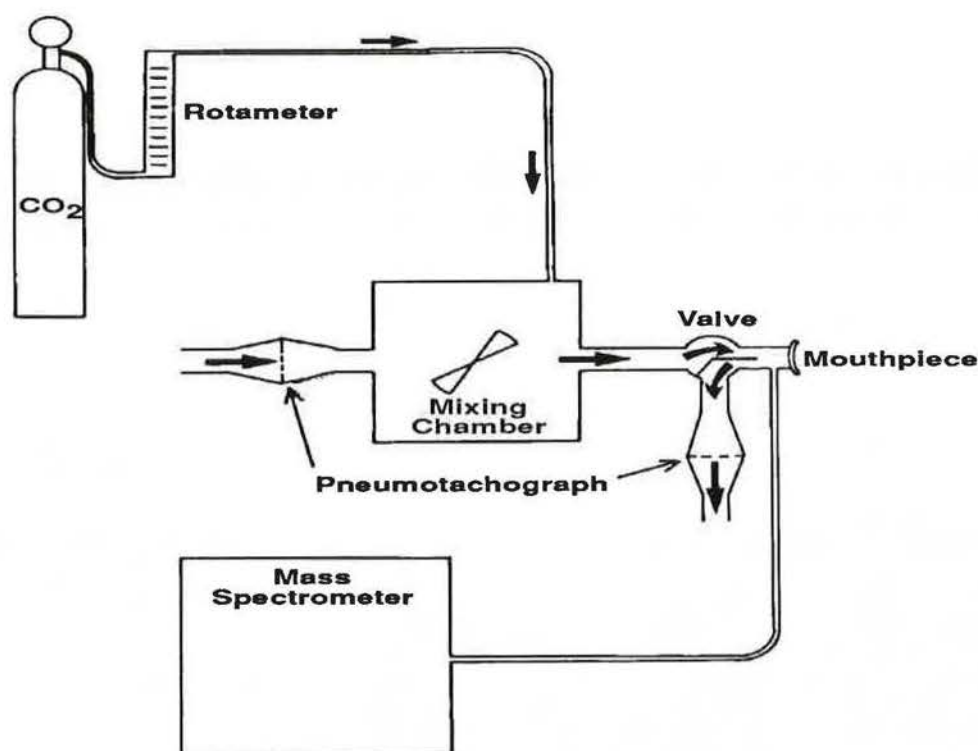
Fig. 1. – The breathing circuit. CO_2 was injected into the inspiratory limb *via* a fan-stirred mixing chamber.

Methods

The experiments were performed on nine healthy male volunteers aged 24–35 yrs. On the day of an experiment the subjects were asked to avoid tea, coffee and cigarettes (one was an occasional smoker). The experiments were always begun at 09.30 h and a light lunch was allowed before the afternoon runs. Six subjects were studied at rest and six during exercise at 50 W, three being studied under both conditions.

For the resting experiments each volunteer attended the laboratory on two days, separated by an interval of at least two weeks. They were seated comfortably in a chair, wore a nose-clip and breathed through a mouthpiece attached to an open respiratory circuit *via* a Rudolph No. 2700 valve, which separated the inspiratory and expiratory flows (fig. 1). Inspiratory flow was measured with a Fleisch No. 4 pneumotachograph attached to a Validyne MP 45 differential pressure transducer. A heated pneumotachograph on the expiratory limb was not used to measure ventilation but, together with the inspiratory pneumotachograph, enabled computer detection of the beginning and end of breaths [12]. Pure CO_2 could be added *via* a rotameter into a 1.2 l fan-stirred mixing chamber on the inspiratory limb. The P_{CO_2} at the mouth was measured continuously with a Centronics MGA 200 mass spectrometer. The signals were recorded on a Gould chart recorder and a Racal FM tape-recorder.

After the subject had been breathing through the apparatus for 10 min, CO_2 was infused into the mixing chamber on the inspiratory limb at flows of 0.2, 0.4, and 0.8 $l \cdot min^{-1}$, allowing 6 min for equilibration at each flow rate. On each day, two such CO_2 responses were performed in the morning, separated by a 10 min rest, and two in the afternoon. One hundred and fifty minutes before the afternoon runs the subject took 100 mg almitrine or placebo according to a randomized, double-blind, crossover schedule. Blood for almitrine levels was collected just before the tablets were taken and just before and just after the afternoon runs.

For the exercise experiments the subject was seated on a Siemens 380B electrically-braked cycle ergometer. Of the six subjects who volunteered for the exercise experiments, three had also been subjects for the resting experiments. After 5 min on the apparatus at rest, the subject was asked to pedal at 60 cycles·min⁻¹ with the work load set at 50 W. After a further 5 min the CO_2 was added *via* the mixing chamber at 0.1, 0.2, 0.4, 0.6 and 0.8 $l \cdot min^{-1}$, allowing 4 min at each flow. Otherwise the protocol was exactly the same as for the resting experiments.

The taped signals were played back and analysed on a PDP 11/23 computer sampling at 100 Hz. Breath-by-breath ventilation and, for the resting experiments, end-tidal P_{CO_2} ($P_{ET}CO_2$) were derived. At rest, $P_{ET}CO_2$ was taken equal to mean alveolar P_{CO_2} ($P_{A}CO_2$). For the exercising experiments the programme performed linear regression on the final half of the expiratory P_{CO_2} profile and extrapolated this line back to the start of expiration. In this way the oscillation of the alveolar P_{CO_2} was approximately reconstructed so that an

Almitrine had no significant effect on the intercept of the resting CO_2 response on the P_{CO_2} axis (fig. 2). Baseline ventilation and end-tidal P_{CO_2} were also unaffected.
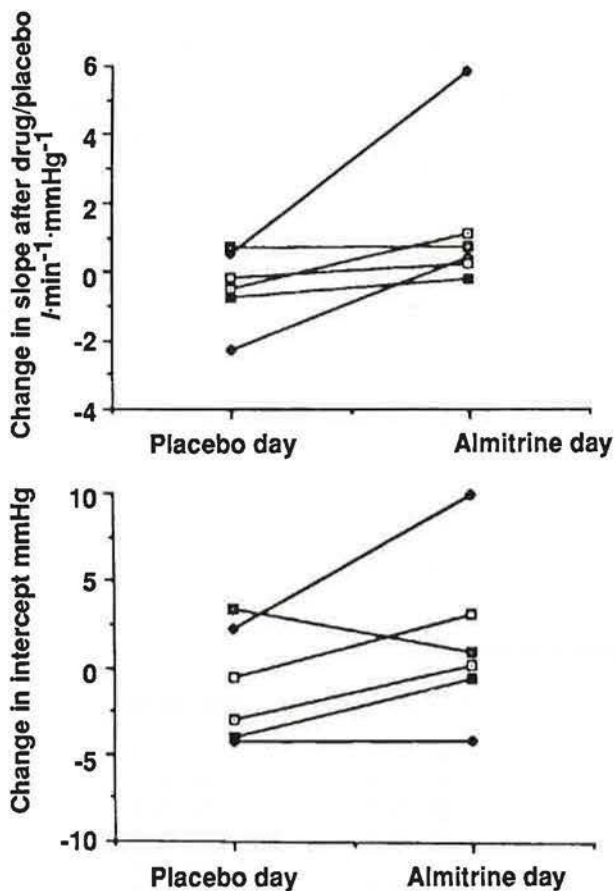

Fig. 2. – Changes in resting steady-state CO_2 response slope (above), and intercept (below) after placebo and after almitrine. Key shows subjects' initials. Almitrine had a significant effect on the slope (p=0.04) but not on the intercept (p=0.11). Key: J: -□- ; A: ◆ ; C: -■- ; N: ● ; D: ■ ; R: -□- .

Table 1. – Plasma almitrine concentrations immediately before and after the afternoon CO_2 responses on the day almitrine was given

	Almitrine concentration ng·ml⁻¹			
	Rest		Exercise	
Subject	Before runs	After runs	Before runs	After runs
A	94	58	154	146
C	30	30	288	221
M	-	-	36	21
SE	-	-	37	21
N	151	114	81	72
S	-	-	27	24
J	131	96	-	-
D	241	118	-	-
R	not assayed		-	-

No almitrine was detectable on any of the placebo days.

estimate of $P_{A CO_2}$ was obtained, a technique that has been validated against directly measured arterial P_{CO_2} [9]. The two morning and the two afternoon runs from each day were merged on a common time base and averaged in 60 s bins. CO_2 responses were constructed by plotting the ventilation from the final minute at each flow rate against $P_{ET CO_2}$ for the resting runs and $P_{A CO_2}$ for the exercising runs; the slopes were obtained by linear regression.

Results

Following placebo four of the six subjects showed a fall in the resting CO_2 response slope, whereas after almitrine five subjects showed an increase in the resting CO_2 response slope on the day almitrine was given (fig. 2). A paired, two-tailed Student's t-test on the log transformed data showed that these results were significant (p=0.04). There was no correlation between individual plasma concentrations of almitrine and the slope changes but levels were only available in five of the six subjects (table 1).
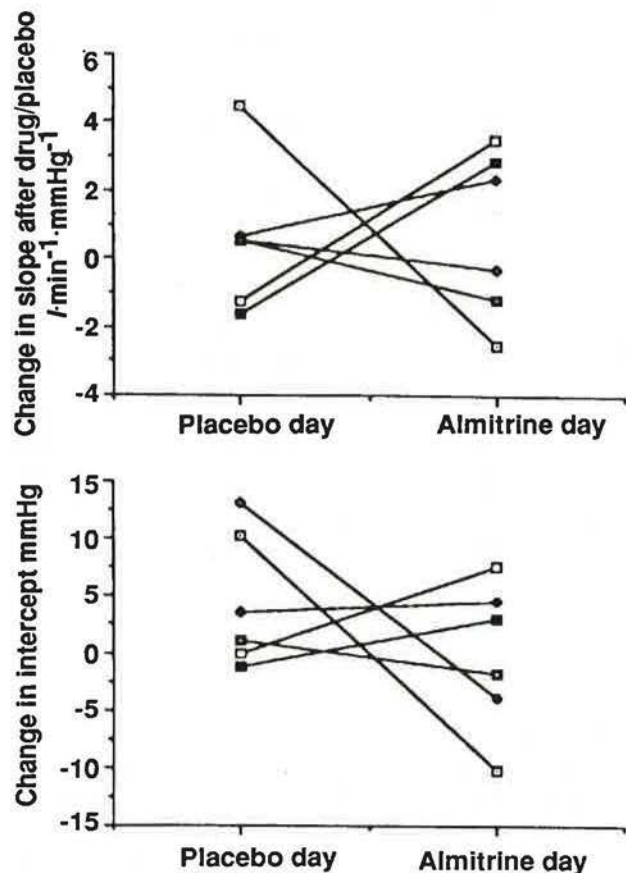

Fig. 3. – Change in exercising steady-state CO_2 response slope (above) and intercept (below) following placebo and almitrine. Key shows subjects' initials. Almitrine had no significant effect on either the slope (p=0.63) or the intercept (p=0.31). Key: A: -□- ; C: ◆ ; M: -■- ; SE: ● ; N: ■ ; S: -□- .

Table 2. – Comparison of the average resting and exercising CO_2 response slopes for all runs performed without almitrine

| | CO_2 response slope $l \cdot min^{-1} \cdot mmHg^{-1}$ | |
| | without almitrine | |
Subject	Rest	Exercise
A	2.58	4.90
C	1.85	3.07
M	-	4.95
SE	-	1.67
N	2.99	5.30
S	-	3.93
J	2.44	-
D	1.69	-
R	1.62	-

The increased slope on exercise is significant (p=0.02, unpaired Student's t-test).

rest and during exercise are compared in table 2. Each of the three subjects who were studied both at rest and during exercise showed increased CO_2 responsiveness during exercise. Taking all six subjects, the average slope was over 80% higher during exercise, an increase which was significant (p=0.02, unpaired, two-tailed t-test after log transformation). For the runs performed after almitrine the average slope was only 35% higher during exercise than at rest. This difference was not significant but fewer responses fell into this category.

The individual response slopes and intercepts are shown in table 3.

Table 3. – Individual CO_2 response slopes ($l \cdot min^{-1} \cdot mmHg^{-1}$) and intercepts on the Pco_2 axis (mmHg)

| | Resting CO_2 response slopes/intercepts | | | |
Subject	Before placebo	After placebo	Before almitrine	After almitrine
J	2.43, 31.2	1.92, 28.2	2.98, 34.8	4.14, 35.0
A	2.79, 35.9	3.33, 38.1	1.61, 32.1	7.50, 42.1
C	1.48, 30.6	2.20, 34.0	1.87, 31.0	2.62, 32.0
N	4.61, 39.5	2.35, 35.2	2.01, 40.9	2.50, 36.8
D	1.91, 30.3	1.14, 26.3	2.29, 33.1	2.08, 32.5
R	1.40, 32.0	1.21, 31.4	2.25, 34.1	2.59, 37.2
Mean	2.44, 33.3	2.03, 32.2	2.17, 32.2	3.57, 35.9
SD	1.19, 3.68	0.81, 4.44	0.47, 3.49	2.05, 3.70

| | Exercising CO_2 response slopes/intercepts | | | |
Subject	Before placebo	After placebo	Before almitrine	After almitrine
A	2.52, 26.5	6.97, 36.7	5.22, 33.9	2.70, 23.8
C	2.57, 20.4	3.24, 24.0	3.40, 23.6	5.78, 28.1
M	4.40, 36.8	4.92, 37.9	5.53, 39.5	4.31, 37.6
SE	1.06, 1.6	1.57, 14.7	2.39, 25.7	2.07, 21.8
N	6.31, 36.3	4.68, 35.1	4.90, 36.1	7.77, 39.0
S	5.19, 33.3	3.94, 33.3	2.66, 26.1	6.20, 33.7
Mean	3.68, 25.8	4.22, 30.3	4.02, 30.8	4.81, 30.7
SD	1.96, 13.4	1.81, 9.10	1.37, 6.5	2.18, 7.20

SD: standard deviation.

During exercise the slopes of the individual responses showed wide variations. Almitrine had no consistent effect on either the slope or the intercept on the Pco_2 axis (fig. 3). Again, there was no effect on baseline ventilation or $Petco_2$. However, some of the subjects showed rather low almitrine levels (table 1) by comparison with those achieved in the resting study.

The average slopes of all the CO_2 responses performed in the absence of almitrine for each subject at

Discussion

The purpose of these experiments was to find out whether almitrine might have a greater effect on the slope of the steady-state ventilatory response to CO_2 than had previously been found in hyperoxia and to see whether the drug might prove a useful tool for studying the ventilatory control mechanisms operating during exercise. The results show that even in relative normoxia the effects of almitrine on the resting CO_2 response are small compared with incidental variation and during exercise there was no detectable effect at all.

The findings at rest are similar to those of other investigators. Using the rebreathing method and administering almitrine intravenously (0.5 $mg \cdot h^{-1} \cdot kg^{-1}$ over 2 h), STRADLING et al. [3] found an insignificant rise in hypercapnic drive. STANLEY et al. [6] also found an insignificant rise after an oral dose of 50 mg but after 100 mg the ventilatory response slope increased by 27%. On the other hand CONNAUGHTON et al. [4] using similar oral doses found no change and even when using a steady-state method, GUILLERM and RADZISZEWSKI [7] found no effect at a dose of 3 $mg \cdot kg^{-1}$, although there was some effect at 5 $mg \cdot kg^{-1}$. At this higher dose there was also a change in normoxic baseline ventilation but at the lower doses used by the other investigators, as in the present work, there was no change. Nevertheless, the smaller doses are certainly sufficient to have an effect on the peripheral chemoreceptor as there were clear increases in hypoxic sensitivity.

In common with the other investigators, we found no correlation between chemosensitivity and plasma almitrine concentration, although some observations suggest that the effect of almitrine on both hypoxic and hypercapnic sensitivity are dose-dependent [6, 7] and in patients with chronic air flow limitation given a single dose there is a correlation between plasma levels and blood gas changes [13]. Plasma almitrine concentrations in our subjects were highly variable ranging from 21–288 $ng \cdot ml^{-1}$. Such wide variations are well recognized [6] and cannot be explained by differences in body weight. Even within subjects there can be considerable variation in the plasma concentration following a single oral dose (table 1).

In the cat, OLIEVIER et al. [5] have studied the peripheral chemoreflex loop in isolation using both the dynamic end-tidal forcing technique and artificial brain stem perfusion. They found that peripheral gain was nearly doubled by almitrine. As the effects of almitrine on chemosensitivity are confined to its action on the peripheral chemoreceptor and as, at rest and in normoxia, the peripheral chemoreceptors only contribute about one third of the respiratory drive [14], the smaller effect seen in man on the overall resting hypercapnic response is to be expected. However, whereas in the present study and the other studies in man no change was seen in the response intercept, OLIEVIER et al. [5] found a marked decrease in the apnoeic threshold. Drawing parallels with similar differences between cat and man in the effects of hypoxia on the CO_2 response, they imply that the discrepancies may simply be due to a species difference.

Even in high doses the effects of almitrine are partly antagonized by pure oxygen [7]. It is surprising then that the effect of almitrine on steady-state CO_2 responsiveness is not greater than that seen in rebreathing. It is worth noting that although "normoxic" steady-state CO_2 responses are performed with the subject breathing air, the increased ventilation brought about by the CO_2 occurs on a background of relatively fixed oxygen consumption, resulting in an element of hyperoxia which may be sufficient to depress the carotid body [15]. However, during the course of our CO_2 responses we observed an average rise in end-tidal Po_2 of only 18 mmHg at rest and 11 mmHg during exercise, increases which would have only a trivial effect on the CO_2 response slope.

During exercise, ventilation and carbon dioxide output are closely matched but our understanding of the mechanisms is poor. A crucial question is whether chemosensitivity to CO_2 is increased during exercise. The work presented here confirms our previous work [9, 10] showing that close to the control point the slope of the CO_2 response is increased during exercise. The results from other investigators have been less consistent [16]. We believe that the discrepancies in the literature may be accounted for by technical differences, in particular the Pco_2 range over which the CO_2 responsiveness is measured – those examining the lower ranges finding an increase. HULSBOSCH et al. [17] came to similar conclusions but were unable to demonstrate a mechanism for the increased sensitivity.

Any increase in chemoreceptor gain might be peripheral or central or both. The possibility that the peripheral chemoreceptors might be having a greater influence on the control of ventilation during exercise is suggested by the observation that oxygen has a greater effect in transiently depressing ventilation during exercise [18] than at rest. Because of this, we anticipated that almitrine might have a much greater effect on CO_2 sensitivity during exercise than at rest, a result which would have confirmed the importance of the peripheral chemoreceptor during exercise. The finding that almitrine had no detectable effect during exercise is less helpful. While this may mean that the carotid body is unimportant in CO_2 chemosensitivity during exercise, an alternative explanation might be that during exercise the peripheral chemoreceptor is already so active that any effect of almitrine is swamped.

References

1. Dhillon DP, Barer G. – Respiratory stimulation by almitrine during acute or chronic hypoxia/hypercapnia in rats. *Bull Eur Physiopathol Respir*, 1982, 18, 751–764.
2. Laubie M, Diot F. – Etude pharmacologique de l'action stimulante respiratoire du S.2620. Role des chémorécepteurs carotidiens et aortiques. *J Pharmacol (Paris)*, 1972, 3, 363–374.
3. Stradling JR, Barnes P, Pride NB. – The effects of almitrine on the ventilatory response to hypoxia and hypercapnia in normal subjects. *Clin Sci*, 1982, 63, 401–404.
4. Connaughton J, Calverley PMA, MacNee W, Critchley JAHA, Flenley DC. – The effects of almitrine on the ventilatory response to hypoxia and hypercapnia in normal man. *Clin Sci*, 1982, 62, 5P.
5. Olievier CN, Berkenbosch A, DeGoede J, Kruyt EW. – Almitrine bismesylate and the central and peripheral ventilatory response to CO_2. *J Appl Physiol*, 1987, 63, 66–74.
6. Stanley NN, Galloway JM, Flint KC, Campbell DB. – Increased respiratory chemosensitivity induced by oral almitrine in healthy man. *Br J Dis Chest*, 1983, 77, 136–146.
7. Guillerm R, Radziszewski E. – Effets ventilatoires chez

l'homme sain d'un nouvel analeptique respiratoire, le S 2620. *Bull Eur Physiopathol Respir*, 1974, 10, 775–791.

8.   Fenn WO, Craig AB. – Effect of CO_2 on respiration using a new method of administering CO_2. *J Appl Physiol*, 1963, 18, 1023–1024.

9.   Cummin ARC, Alison J, Jacobi MS, Iyawe VI, Saunders KB. – Ventilatory sensitivity to inhaled carbon dioxide around the control point during exercise. *Clin Sci*, 1986, 71, 17–22.

10.   Jacobi MS, Iyawe VI, Patil CP, Cummin ARC, Saunders KB. – Ventilatory responses to inhaled carbon dioxide at rest and during exercise. *Clin Sci*, 1987, 73, 177–182.

11.   Cummin ARC, Jacobi MS, Patil CP, Saunders KB. – Effect of almitrine on the CO_2 response in man by a steady state method. *Bull Eur Physiopathol Respir*, 1987, 23, 408S.

12.   Cummin ARC, Patil CP, Jacobi MS, Saunders KB. – Effect on ventilation of carbon dioxide delivered in early inspiration in man. *Bull Eur Physiopathol Respir*, 1987, 23, 335–338.

13.   Bromet N, Aubert Y, Baune A, Courte S, Guillaudeux J. – A pharmacokinetic study of almitrine. *Rev Fr Mal Respir*, 1980, 147–154.

14.   Bellville JW, Whipp BJ, Kaufman RD, Swanson GD, Aqleh KA, Wiberg DM. – Central and peripheral chemoreflex loop gain in normal and carotid body-resected subjects. *J Appl Physiol: Respirat Environ Exercise Physiol*, 1979, 46, 843–853.

15.   Goode RC, Brown EB, Howson MG, Cunningham DJC. – Respiratory effect of breathing down a tube. *Respir Physiol*, 1969, 6, 343–359.

16.   Flenley DC, Warren PM. – Ventilatory responses to O_2 and CO_2 during exercise. *Ann Rev Physiol*, 1983, 45, 415–426.

17.   Hulsbosch MAM, Binkhorst RA, Folgering HTh. – Interaction of CO_2 and positive and negative exercise stimuli on the ventilation in man. *Pflügers Arch*, 1982, 394, 16–20.

18.   Whipp BJ. – The control of exercise hyperpnoea. *In:* The control of breathing in man. B.J. Whipp ed., Manchester University Press, 1987, pp. 87–118.

Effet de l'almitrine sur la réponse ventilatoire en état stable à l'égard du CO_2, au repos et à l'effort, chez l'homme. A.R.C. Cummin, M.S. Jacobi, C.P. Patil, R.J. Telford, C.N. Morgan, K.B. Saunders.

RÉSUMÉ: L'almitrine est un outil potentiel pour tester le rôle physiologique des chémo-récepteurs périphériques. Les effets de l'almitrine sur la chémo-sensibilité au CO_2 ont été étudiés au repos et au cours d'un effort léger, en utilisant une technique d'influx constant qui évite l'hyperoxie des méthodes de rebreathing. La réponse ventilatoire en steady state à l'égard de CO_2 a été mesurée dans deux groupes de six hommes normaux, avant et 150 minutes après la prise de 100 mg de bismesylate d'almitrine per os ou de placebo. Un groupe a été étudié au repos, l'autre pendant un effort de 50 watts à la bicyclette. Le groupe au repos a démontré une augmentation significative de la pente de réponse au CO_2 après almitrine, par comparaison avec le placebo; mais il n'y a pas eu de modification significative dans l'interception de la réponse. Pendant l'effort, les résultats individuels furent très variables; et après almitrine aucune modification significative n'a été trouvée, ni dans la pente de la réponse, ni dans l'interception. La ventilation de contrôle n'a pas été affectée par l'almitrine dans aucun des groupes. Même en l'absence d'une hyperoxie marquée, l'effet de l'almitrine sur la sensibilité au CO_2 au repos est faible. Le manque d'effet à 50 watts plaide contre tout rôle important attribuable aux chémo-récepteurs périphériques au cours d'un effort léger. D'autres interprétations sont toutefois possibles. *Eur Respir J., 1990, 3, 693–698.*