Ventilatory and metabolic responses to ambient hypoxia or hypercapnia in rats exposed to CO hypoxia

HENRY GAUTIER, CRISTINA MURARIU, AND MONIQUE BONORA
Atelier de Physiologie Respiratoire, Faculté de Médecine Saint-Antoine, 75012 Paris, France

Gautier, Henry, Cristina Murariu, and Monique Bonora. Ventilatory and metabolic responses to ambient hypoxia or hypercapnia in rats exposed to CO hypoxia. J. Appl. Physiol. 83(1): 253–261, 1997.—We have investigated at ambient temperatures (Tamb) of 25 and 5°C the effects of ambient hypoxia (Hxam, fractional inspired O2 = 0.14) and hypercapnia (fractional inspired CO2 = 0.04) on ventilation (V), O2 uptake (VO2), and colonic temperature (Tc) in 12 conscious rats before and after carotid body denervation (CBD). The rats were concomitantly exposed to ambient hypoxia (HxCO; fractional inspired CO = 0.03–0.05%), which decreases arterial O2 saturation by ~25–40%. The results demonstrate the following: 1) At Tamb of 5°C, in both intact and CBD rats, VO2 is larger when Hxam or CO2 is associated with HxCO than with normoxia. At Tamb of 25°C, this is also the case except for CO2 in CBD rats. 2) At Tamb of 5°C, the changes in VO2 and Tc seem to result from additive effects of the separate changes induced by Hxam, CO2, and HxCO. It is concluded that, in conscious rats, central hypoxia does not depress respiratory activity. On the contrary, particularly when VO2 is augmented during a cold stress, both VO2 during HxCO and the ventilatory responses to Hxam and CO2 are increased. The mechanisms involved in this relative hyperventilation are likely to involve diencephalic integrative structures.

thermoregulation; chemoreceptors; hypoxic hypometabolism; control of breathing; shivering; carbon monoxide

In the absence of peripheral chemoreceptor stimulation, hypoxia may affect respiratory activity by a direct effect on the brain. This has been demonstrated in animals with isolated carotid body perfusion or with carotid body denervation (CBD) exposed to ambient hypoxia (Hxam, decreased inspiratory P02) and in intact animals during inhalation of low concentrations of CO. The changes in respiratory activity induced by brain hypoxia seem to vary with the experimental conditions. In anesthetized animals, a depressant effect has usually been reported (3, 6, 24, 32), whereas, in conscious animals, brain hypoxia consistently induces an increase in ventilation (V; see Ref. 29). However, independent of the effects of brain hypoxia on breathing (depression or stimulation), the respiratory responses to hypercapnia and carotid sinus nerve stimulation during brain hypoxia appear to be unchanged (23, 29, 32). These results suggest that, under these conditions, the integration of the chemosensory inputs by the brain stem is unaltered.

We have observed in a previous study carried out in unanesthetized intact rats (13) that central hypoxia resulting from the inhalation of a low concentration of CO induces an increase in V relative to O2 consumption (VO2), particularly when the animals were studied at low ambient temperatures (Tamb). Following these results and using the same experimental conditions, the present study was designed to test the hypothesis that the ventilatory responses to Hxam and CO2 are increased when associated with central hypoxia induced by CO exposure. The experiments were carried out at Tamb of 25 and 5°C. Furthermore, the contribution of the peripheral chemoreceptors to the changes observed was assessed by studying rats before and after CBD.

METHODS

Animals

Experiments were performed in groups of 12 male Wistar rats, ~2 mo old, with an average body weight of 230 g at the beginning of the study. They were caged in an animal room in groups of three at Tamb of 23–25°C for at least 2 wk before the experiments. They were fed with a commercial rat chow and tap water ad libitum.

Measurements

After the rats were weighed, they were placed in a calorimeter. Flow rates through the calorimeter of the various gas mixtures used were measured with a rotameter and kept at 1.5 l/min. The concentrations of O2 (Beckman OM 14), CO2 (Beckman LB 2), and CO (Cosma Diamant 6000, Igny, France) in the inflow or outflow gas streams were continuously recorded on a chart recorder and were used to compute VO2 with the open-circuit method. The analyzers were repeatedly calibrated by using standard gases of known composition. V, colonic temperature (Tc), and Tamb (calorimeter) were monitored as previously described (13). In all studies, VO2, Tc, Tamb, and V were computed at 10-min intervals. In the experiments carried out at low Tamb, the shivering activity was continuously monitored and averaged over 10-min periods (13).

In each of the following protocols, the rats were studied both before (intact) and after CBD. Under a surgical plane of halothane anesthesia (4% for induction, then 1% for maintenance, in O2), the carotid sinus nerves were exposed by using an operating microscope and were subsequently sectioned at their junctions with the glossopharyngeal nerves. Experiments were carried out within 5–15 days after the surgery.

Ten of the CBD rats described above, as well as 10 additional intact animals, were prepared for arterial blood sampling. During a brief period of halothane anesthesia, an indwelling catheter was inserted into the left carotid artery. The rats were then returned to their cages, and blood-gas measurements were made during the experiments carried out 1–3 days later.

Protocols

Four different protocols were used. The first protocol, which involved measurements of arterial blood gases, was carried out at Tamb of 25°C. The other three protocols were performed in each animal on different days at Tamb of 25°C (normothermia) and 5°C (hypothermia). These experiments were carried out with hypoxia [fractional inspired O2 (FIO2) of 14%], with hypercapnia [fractional inspired CO2 (FICO2) of 4%] and with CO [fractional inspired CO (FICO) of either 0.03 or

http://www.jap.org 0161-7567/97 $5.00 Copyright © 1997 the American Physiological Society 253
0.05% in ambient air] at Tam of 5 and 25°C, respectively. For Tam of 5°C, the FICO of 0.03% was selected because in a previous study we have shown that, under these conditions, there were marked changes in VO₂, TCO₂, and V/VO₂ (13). Because at Tam of 25°C, there were no consistent changes in VO₂ or TCO₂ with FICO of 0.03%, in the present experiments performed at this Tam, we have selected a higher concentration of inspired CO (0.05%).

Protocol 1: Arterial blood gases during hypoxia. Animals were moderately restrained in a small transparent container maintained at Tam of 25°C. The container was flushed with gases of the same composition as detailed in protocol 3, part 2. A sample of 0.2 ml of arterial blood was collected after 30 min of exposure to normoxia (Nx), 30 min of CO (0.03 or 0.05% in ambient air), 30 min of CO and hypoxia, and finally 30 min of CO again. Blood samples were immediately analyzed with a Radiometer ABL 500 blood-gas analyzer for arterial PO₂ (PaO₂), arterial PCO₂ (PaCO₂), and arterial pH, and with a Radiometer OSM3 set for rat blood for arterial O₂ content (CaO₂), and arterial saturation of O₂ and CO (SaO₂ and SaCO₂, respectively).

Protocol 2: Ventilatory and metabolic responses to HxCO. Animals were initially studied during a control period of Nx for at least 30 min. During this period, they settled down, and the different variables studied became stable. Thereafter, animals were exposed to CO mixed with ambient air for 90 min followed by 10–15 min of 100% O₂.

Protocol 3: Ventilatory and metabolic responses to hypoxia. After the control period of Nx, animals were exposed sequentially to 30-min periods of either 1) Nx, hypoxia, and again Nx or 2) CO, CO and hypoxia, and again CO, followed by 10–15 min of 100% O₂.

Protocol 4. Ventilatory and metabolic responses to hypercapnia. After the control period of Nx, animals were exposed sequentially to 30-min periods of either 1) Nx, then hypercapnia and Nx, or 2) CO, CO and hypercapnia, and again CO, followed by 10–15 min of 100% O₂.

Statistics

Statistical analyses were carried out by using BioMedical Data Package programs. A two-way analysis of variance was performed to assess the significance of the changes observed in the variables studied. If a significant (P < 0.05) F-ratio was found, then specific statistical comparisons were made. Dunnett's test was used to assess the significance of the sequential changes induced by 10–90 min of HxCO compared with the control data that were obtained after 30 min of Nx. The same test was used to assess the changes induced by 10–30 min of hypoxia or hypercapnia compared with the control data that were obtained after either 60 min of Nx or 30 min of HxCO. Bonferroni's test was used to compare the effects of 30 min of hypoxia or hypercapnia administered after either 60 min of Nx or during CO hypoxia. A paired t-test was also used when appropriate. Statistical significance was accepted at the P < 0.05 level. The data are presented as means ± SE.

RESULTS

Blood Gases

As shown in Fig. 1, in both intact and CBD rats, CO inhalation induced a progressive increase of SaCO₂ and, correlatively, a progressive decrease of SaO₂, which after 60 min of HxCO and 30 min of Hxam reached minimums of ~70 and 58% with FICO of 0.03 and 0.05%, respectively (Fig. 1, A and B, respectively) and did not change thereafter. During Hxam, PaO₂ decreased to reach nadirs of ~60 and 48 Torr in intact and CBD rats, respectively.

Fig. 1. Average values of arterial PO₂ (PaO₂), arterial PCO₂ (PaCO₂), arterial O₂ saturation (SaO₂), and arterial CO saturation (SaCO₂) in rats before (intact; closed symbols) and after carotid body denervation (CBD; open symbols). At ambient temperature (Tam) of 25°C, animals were exposed to normoxia (Nx; circles), next to CO hypoxia (HxCO) with 0.03 or 0.05% CO in room air (A and B, respectively; squares), then to HxCO and ambient hypoxia (Hxam; fractional inspired O₂ (FICO) = 0.14; triangles), and finally to HxCO alone.
Normothermia. Compared with control Nx, exposure to HxCO did not induce any significant change in V and VO₂ in both intact and CBD rats (Fig. 2). A significant decrease in Tc was observed only in the intact rats toward the end of HxCO exposure.

Hypothermia. As shown in Fig. 3, when intact and CBD rats were exposed to HxCO, there was no significant change in V. In contrast, VO₂ decreased markedly and by the same extent during HxCO in both intact and CBD rats. It follows that after 90 min of CO exposure, V/VO₂ was significantly increased (P < 0.001) from 23 ± 1 to 29 ± 1 ml/ml in intact rats and from 20 ± 1 to 28 ± 1 ml/ml in CBD rats. Tc decreased markedly in both intact and CBD rats. Although shivering intensity did not change significantly in intact animals, it progressively decreased in CBD rats, the decrease amounting to 20% after 90 min of HxCO exposure.

Hxam and HxCO

Normothermia. In intact animals, when Hxam was associated with HxCO, the V was significantly greater than during Hxam alone, but the decreases in VO₂ were similar in both situations (Fig. 4A). As a result, the V/VO₂ was significantly greater with Hxam combined with HxCO than with Hxam alone (57 ± 3 and 42 ± 2 ml/ml, respectively; P < 0.01). In CBD animals, when Hxam was combined with HxCO, there was a small but significant increase in V, whereas with Hxam alone the V did not change significantly (Fig. 4B). In both situations, similar decreases in VO₂ were observed. As in intact animals, the V/VO₂ was significantly greater with Hxam combined with CO than during Hxam alone (38 ± 1 and 31 ± 1 ml/ml, respectively; P < 0.01).

Hypothermia. In intact animals, V did not change significantly during the Hxam exposure, regardless of whether or not the rats were concomitantly exposed to HxCO (Fig. 5A). However, after 30 min of Hxam alone, V was significantly greater than when it was associated with HxCO. VO₂ decreased markedly during Hxam and even more significantly when this was associated with HxCO. As a result, V/VO₂ was significantly greater during Hxam and HxCO than during Hxam alone (34 ± 1 and 30 ± 1 ml/ml, respectively; P < 0.05). During Hxam, Tc decreased progressively but partially recovered during the subsequent return to Nx. Such recovery, however, was absent in the HxCO experiments (Fig. 5A). At the onset of Hxam, shivering intensity decreased, but it...
Fig. 4. \( V, \dot{V}_{\text{O}_2}, \text{and } T_c \) in rats before (A) and after CBD (B). At \( T_{\text{am}} \) of 25°C, animals were exposed first to Nx (●), next to either Nx or 0.05% CO in room air (\( H_{\text{XCO}} \) ○), then to \( H_{\text{Xam}} \) (\( F_i \text{O}_2 = 0.14 \)), and finally again to either Nx or \( H_{\text{XCO}} \). *Values in \( H_{\text{Xam}} \) that are significantly different from previous measurements in Nx or \( H_{\text{XCO}} \), \( P < 0.05 \). \( \star \) Values after 30 min of \( H_{\text{Xam}} \) that are significantly different during \( H_{\text{XCO}} \) compared with \( H_{\text{Xam}} \) alone, \( P < 0.05 \).

Fig. 5. \( V, \dot{V}_{\text{O}_2}, T_c, \) and shivering intensity in rats before (A) and after (B) CBD. At \( T_{\text{am}} \) of 5°C, animals were first exposed to Nx (●), next to either Nx or 0.03% CO in room air (\( H_{\text{XCO}} \) ○), then to \( H_{\text{Xam}} \), and finally again to either Nx or \( H_{\text{XCO}} \). *Values in \( H_{\text{Xam}} \) that are significantly different from the previous measurement in Nx or \( H_{\text{XCO}} \), \( P < 0.05 \). \( \star \) Values that are significantly different after 30 min of \( H_{\text{XCO}} \) compared with Nx or after 30 min of \( H_{\text{Xam}} \) with \( H_{\text{XCO}} \) compared with \( H_{\text{Xam}} \) alone, \( P < 0.05 \).
returned to control values thereafter. Such recovery was not observed in the HxCO experiments.

In CBD animals, an initial drop in $V$ was observed with both Hxam and Hxam with HxCO, with a gradual recovery by 30 min of hypoxia (Fig. 5B). $V_O_2$ decreased markedly with Hxam and even more with Hxam and HxCO. As a result, $V/O_2$ was significantly greater with Hxam and HxCO than with Hxam alone (30 $\pm$ 2 and 26 $\pm$ 1 ml/ml, respectively; $P < 0.05$). $T_c$ and shivering activity exhibited the same response as in intact animals. Similarly, shivering was significantly less with Hxam and HxCO than with Hxam alone.

**Hypercapnia and HxCO**

Normothermia. In intact animals, CO$_2$ with or without HxCO induced a marked increase in $V$ while $V_O_2$ did not change significantly during hypercapnia (Fig. 6A). As a result, $V/O_2$ was significantly increased with CO$_2$ and HxCO compared with normoxic CO$_2$ (54 $\pm$ 2 and 47 $\pm$ 2 ml/ml, respectively; $P < 0.05$). During both normoxic hypercapnia and hypercapnia with HxCO, $T_c$ decreased similarly, although not significantly. In CBD animals, the ventilatory response to CO$_2$ was the same in Nx as during HxCO (Fig. 6B). Also $V_O_2$, $V/O_2$, and $T_c$ were not significantly different under both conditions of hypercapnia.

Hypothermia. In intact animals, $V$ increased progressively during hypercapnia, and the level reached after 30 min was not significantly different in HxCO than in Nx. $V_O_2$ decreased significantly at the onset of hypercapnia and more markedly when CO$_2$ was associated with HxCO. Under both conditions, $V_O_2$ partially recovered during the last 20 min of hypercapnia (Fig. 7A). As a consequence, $V/O_2$ was significantly higher with CO$_2$ during HxCO than during Nx (40 $\pm$ 1 and 33 $\pm$ 1 ml/ml, respectively; $P < 0.01$). $T_c$ decreased significantly only during hypercapnia with HxCO. In contrast, shivering decreased significantly at the onset of hypercapnia, with a subsequent partial recovery both during normoxic hypercapnia and hypercapnia combined with HxCO (Fig. 7A).

In CBD animals, the ventilatory response to CO$_2$ was about the same in HxCO as in Nx (Fig. 7B). As $V_O_2$ decreased substantially more with HxCO, $V/O_2$ was significantly greater in the former situation (36 $\pm$ 2 and 28 $\pm$ 1 ml/ml, respectively; $P < 0.01$). $T_c$ decreased significantly only during hypercapnia with HxCO, while shivering did not change significantly under both experimental conditions.

**DISCUSSION**

The discussion of the results of the present study must take into account the fact that in small mammals, such as rats, the ventilatory responses to hypoxia and hypercapnia may reflect the interaction of two opposing effects, namely, an increase in chemoreceptor drive and a decrease in metabolism. As recently pointed out, this is particularly prominent at low $T_a$ (11, 25). It follows that the ventilatory response to a given stimulus should be analyzed not only in terms of changes in absolute $V$ but also in terms of changes in $V$ relative to the $V_O_2$ ($V/O_2$). With this in mind, the results of the
The present study may be summarized as follows. 1) In intact animals, at $T_{am}$ of both 25 and 5°C, the ventilatory responses to CO$_2$ and HXam are increased during HXCO. 2) In CBD animals, even though the ventilatory response to HXam is markedly reduced compared with intact rats, it is nevertheless enhanced when associated with HXCO. The ventilatory response to CO$_2$ is also increased with HXCO at $T_{am}$ of 5°C, whereas it remains unaffected at $T_{am}$ of 25°C. 3) The changes in metabolic responses to cold, and, therefore, in $T_c$ observed during HXCO, HXam, and hypercapnia in intact and CBD rats are in general agreement with several previous studies (13, 15, 26). 4) The latter responses seem to result from the additive effects of HXam and CO$_2$ with HXCO.

**Ventilatory Response to HXCO**

We have reported in a previous study (13) that there are no appreciable changes in V in rats exposed to normothermia with FICO of 0.03%, which causes a decrease in CaO$_2$ of 25%. This is confirmed by the present findings obtained at a higher level of FICO (0.05%), which induces a 40% decrease in CaO$_2$. It should be noted, however, that rats may respond to HXCO differently from other animal species, such as goats (27) and cats (13), that characteristically exhibit hyperventilation (hypoxic tachypnea) with a similar decrease in CaO$_2$ to that observed in the present study. Recently, a maximal increase in V of 275% has been observed in anesthetized rats exposed to HXCO which, like the present study, induced a 40% decrease in CaO$_2$ (10). However, the latter results are at variance with those of Matsuoka et al. (20). They showed that in conscious rats in which hemoglobin concentration ([Hb]) was acutely reduced by ~50%, there was a maximal increase in V of only 30%.

In hypothermia, the present results confirm those of our previous study, showing that V does not change significantly during HXCO while VO$_2$ decreases markedly (13). This absence of coupling of V to VO$_2$ led us to postulate the existence of an additional V-stimulating factor that counteracts the ventilatory effects of HXCO-induced hypometabolism. Because the present study shows that identical results are found in CBD animals, it follows that this V-stimulating factor originates centrally (see Integrated Effects of HXCO and HXam or CO$_2$ on V).

**Effects of HXCO on the Ventilatory Response to HXam and CO$_2$**

The effects of brain hypoxia on the control of breathing have been the object of many previous studies. According to recent reviews, brain hypoxia is believed to primarily promote ventilatory depression (3, 6). Such depressant effects, which are consistently observed in anesthetized preparations in the absence of peripheral chemoreceptor stimulation, have been advanced to explain the biphasic nature of the ventilatory response to hypoxia observed in intact animals. However, in the past 20 years, several studies have clearly shown that, in unanesthetized animals, isolated brain hypoxia induced by 1) HXam after peripheral chemodenervation, 2) HXCO in intact animals, or 3) systemic hypoxia and
selective carotid perfusion with normoxic blood characteristically results in hyperventilation. Furthermore, when the carotid bodies are concomitantly stimulated by hypoxia, the resulting increase in \( V \) is about the same whether the brain is hypoxic or normoxic (4, 29). In contrast to the above results, the present study shows that the ventilatory responses to \( Hxam \) and \( CO_2 \) are enhanced during \( HxCO \), even though brain hypoxia per se had no effect on control \( V \).

Several studies similar to ours have dealt with the ventilatory response to \( Hxam \) and \( CO_2 \) when \( CaO_2 \) was decreased by experimental anemia or by \( HxCO \) in rats and cats. With a decrease in [Hb] of \( \approx 50\% \), the ventilatory responses to both \( Hxam \) and \( CO_2 \) were found unchanged (1). However, it should be noted that \( V_O_2 \) was not measured in these studies. Similarly, in goats in which [Hb] was decreased \( > 60\% \), the response to steady-state (6 min) \( Hxam \) or \( CO_2 \) was unaffected (28). In both of the above studies, the ventilatory responses to \( Hxam \) or \( CO_2 \) were investigated 3–5 days after the induction of anemia. This delay may be critical, as recently shown in a study on rats. Three hours after induction of anemia ([Hb] reduced by 50%), the \( V \) was increased while \( V_O_2 \) and \( T_c \) were decreased. After 3 days, however, all of these variables had returned to control values (20).

The effects of \( HxCO \) on the ventilatory response to \( CO_2 \) have been investigated in goats and cats. In anesthetized goats, the ventilatory response to \( CO_2 \) studied with the rebreathing method was not consistently changed (27). In anesthetized, curarized, CBD, and vagotomized cats, the phrenic response to \( CO_2 \) was not blunted as expected and, in fact, may have been accentuated by \( HxCO \) (22). In another study using the same experimental approach (23), it was found that the response of the phrenic neurogram to supramaximal carotid sinus nerve stimulation was unaffected even during severe hypoxemic respiratory depression. These studies suggest that the processing by the respiratory centers of the central and peripheral afferent information is unchanged by central hypoxemia. In all of the above studies, \( CaO_2 \) was reduced by \( \approx 50\% \) as a result of the inhalation of \( 0.5–1.0\% CO \) in 40% oxygen, with \( PaO_2 \) maintained well above 150 Torr. In contrast, a much lower concentration of \( O_2 \) (0.03–0.05%) in ambient air was used in the present study, resulting in no change in \( PaO_2 \) (see Fig. 1). Conceivably, for a given reduction in \( CaO_2 \) by \( HxCO \), the respiratory control mechanisms may be affected differently in the presence of a higher \( PaO_2 \). Also, the present results are in agreement with those of a previous investigation in cats exposed to \( CO \) in room air in which we also found an increase in the ventilatory response to \( CO_2 \) (14). Similarly, in conscious CBD cats exposed to a moderate level of \( Hxam \) (\( F_{CO_2} = 0.13 \)), the ventilatory response to \( CO_2 \) was significantly higher than that observed during \( Nx \) (12).

Finally, it may be expected that prolonged exposure to \( HxCO \) should have a time-related effect on gas exchange because of the increase in carboxyhemoglobin between 30 and 60 min (see Fig. 1). Such time dependency is clearly seen on \( V_O_2 \) under hypothermic conditions during either \( HxCO \) alone or when associated with \( Hxam \) or \( CO_2 \). The increase with time in the ventilatory response to \( Hxam \) and \( CO_2 \) may, however, be modulated by several factors: 1) the progressive decrease in \( V_O_2 \) in hypothermia that may counteract and mask the relative increase in ventilatory stimulation; 2) the partial recovery in \( V_O_2 \) between 10 and 30 min, as seen during \( CO_2 \) in hypothermia; and 3) a possible delayed effect of \( HxCO \) on \( V \), even when the level of carboxyhemoglobin is maintained constant, as observed in ponies by Lowry et al. (18).

Integrated Effects of \( HxCO \) and \( Hxam \) or \( CO_2 \) on \( V \)

The potentiation of the ventilatory responses to \( Hxam \) and \( CO_2 \) by \( HxCO \) may originate centrally and/or peripherally at chemoreceptor level. The role of the carotid chemoreceptors is probably small, because the increase in chemosensitivity to \( Hxam \) and \( CO_2 \) during \( HxCO \) persists after CBD, even though the overall ventilatory responses are predictably attenuated. Furthermore, it is generally admitted that \( CO \) per se does not significantly stimulate the carotid chemoreceptors, even though it has been recently shown that in vitro cat preparations, the carotid chemosensory response to hypoxia is enhanced by \( CO \). However, this is observed only with \( PCO_2 \) > 140 Torr (17). Moreover, it has been shown that the increase in \( V \) that is observed in goats and cats during \( HxCO \) persists after CBD (16, 27). The present results, however, cannot exclude a possible role of the aortic or other extracarotid chemoreceptors, which could be stimulated by \( HxCO \).

The nature of the central mechanisms that mediate the increase in \( V/V_O_2 \) during \( HxCO \) in hypothermia and the increased responsiveness to \( Hxam \) or \( CO_2 \) during \( HxCO \) remains speculative. Whereas the central depressing effects of hypoxia described in anesthetized animals are usually assumed to occur at the pontomedullary level (7), the central stimulatory effects of \( Hxam \) and \( HxCO \), which elicit rapid shallow breathing in conscious animals, have generally been attributed to stimulation of structures rostral to the brain stem, particularly at the level of the diencephalon (16, 31). Furthermore, according to Gallman and Millhorn (9), in peripherally chemodenervated cats there is a long-lasting facilitatory effect on respiration after hypoxia, which probably involves also the diencephalon. Finally, there is recent evidence that \( Hxam \) may stimulate caudal hypothalamic neurons, whose basal discharge is correlated with respiratory activity and, interestingly, the stimulation elicited by \( Hxam \) does not require any input from the peripheral chemoreceptors (5). Accordingly, it can be argued that these mechanisms may also have a role in the present study, accounting for the observed interactions of \( HxCO \) and the enhanced ventilatory responses to \( Hxam \) and \( CO_2 \). However, most of the above studies, in which the increase in respiratory activity during hypoxia has been attributed to diencephalic structures, have involved \( Hxam \) and not \( HxCO \). The effects of \( HxCO \) may be different from those of \( Hxam \), and, in fact, appear to be multifactorial. In addition to a decrease in \( CaO_2 \) and the shift to the left of the oxyhemoglobin dissociation curve during \( HxCO \), several other factors appear to contribute to the increased ventilatory activity observed in conscious animals exposed to \( HxCO \).
tion curve, HxCO may markedly affect cerebral blood flow. Furthermore, it has been recently suggested that CO acts as a neural messenger in a manner very similar to nitric oxide (21).

Effects of HxCO on the Metabolic Responses to Hxam and CO2

The present results confirm previous studies showing that in rats, especially during cold exposure, Hxam and HxCO, and to a smaller degree CO2, may affect heat production [shivering and nonshivering thermogenesis (NST)], V˙O2, and hence Tc (13, 15, 25, 26). In addition, the present results show that in both intact or CBD rats, hypometabolism and hypothermia resulting from exposure to either Hxam or HxCO are increased in an additive manner when Hxam and HxCO are administered concomitantly. The same additive effects, although of a smaller magnitude, are observed when HxCO is associated with CO2.

The hypometabolism during hypoxia results from the inhibition of shivering and/or NST, as shown in a previous study (13). Thus, it appears that, in intact animals, shivering is not affected during HxCO. Accordingly, the hypometabolism must result entirely from an inhibition of NST. In contrast, in CBD animals exposed to HxCO, our results show that shivering is significantly depressed. The mechanism of this unexpected finding is not clear. Indeed, the elimination of any carotid body stimulation cannot explain this result as it is generally agreed that, shivering is inhibited rather than stimulated by carotid body (33). Furthermore, CO does not significantly stimulate carotid body. As initially suggested by von Euler (33), shivering may be potentially affected by several factors that are related to both chemoreceptor and baroreceptor functions. The present results suggest that, during HxCO in CBD compared with intact animals, changes in such factors (e.g., hypoxia, hypercapnia, blood pressure) may secondarily affect shivering. The role of these factors, however, cannot explain the fact that, in intact as well as in CBD rats, shivering is more inhibited when Hxam is associated with HxCO compared with HxCO alone. Because no such interaction is observed between CO2 and HxCO, it may be argued that the magnification of the effects of Hxam by HxCO could be related to the interaction between PaCO2 and CaO2 under these conditions. Clearly, further studies are needed to fully account for these results.

The present results confirm previous reports (11) showing that the effects of Hxam and CO2 on body temperature regulation are probably mediated centrally, as they persist after CBD. The interactions of HxCO, Hxam, or CO2 with body temperature probably involve the same structures. Even though the mechanisms involved in the reduction of thermogenesis are not completely understood, it is now generally agreed that, particularly during cold exposure, hypoxia and hypercapnia lower the body temperature set point, which is probably controlled at the level of the diencephalon (11). This notion is supported by the fact that the thermosensitivity of the preoptic neurons is affected by Hxam, and CO2 (30). In this connection, it should be emphasized that the diencephalon appears to have a pivotal role in the modulation by brain hypoxia (HxCO) of both the ventilatory and the metabolic responses to Hxam and CO2. This is supported by a recent study (19) showing that in anesthetized rats the depressant interaction between hypothermia and hypoxia, which results in inhibition of respiration, was eliminated after lesions in the posterior hypothalamic area.

In conclusion, the present results indicate that brain hypoxia induced by HxCO in conscious rats does not elicit ventilatory depression. On the contrary, it potentiates the ventilatory responses to Hxam and to CO2 and accentuates their hypometabolic effects. The observed responses are not qualitatively affected by CBD and are likely to involve diencephalic structures. The latter probably integrate the interactions between the control of V and the control of metabolism, and hence body temperature, in response to changes in oxygenation and/or CO2.

Wethank M. Gras for typing the manuscript, J. Chandelier for art work, and D. Billet for performing blood-gas analyses.

Address for reprint requests: H. Gautier, Faculte´d eM e´decine Saint-Antoine, 27 rue Chaligny, 75012 Paris, France.

Received 8 May 1996; accepted in final form 14 March 1997.

REFERENCES