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SHORT COMMUNICATION

Ventilatory Response to Sustained Hypoxia in Carotid Body Denervated Rats

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Summary

Hypoxia stimulates ventilation, but when it is sustained, a decline in the ventilatory response is seen. The mechanism responsible for this decline lies within the CNS, but still remains unknown. In this study, we attempted to elucidate the possible role of hypoxia-induced depression of respiratory neurons by comparing the ventilatory response to hypoxia in intact rats and those with denervated carotid bodies. A whole-body plethysmograph was used to measure tidal volume, frequency of breathing and minute ventilation (V_E) in awake and anesthetized intact rats and rats after carotid body denervation during exposure to hypoxia (F_IO_2 0.1). Fifteen-minute hypoxia induced an initial increase of V_E in intact rats (to 248 % of control ventilation in awake and to 227 % in anesthetized rats) followed by a consistent decline (to 207 % and 196 % of control V_E, respectively). Rats with denervated carotid bodies responded with a smaller increase in V_E (to 134 % in awake and 114 % in anesthetized animals), but without a secondary decline (145 % and 129 % of control V_E in the 15th min of hypoxia). These results suggest that afferentation from the carotid bodies and/or the substantial increase in ventilation are crucial for the biphasicity of the ventilatory response to sustained hypoxia and that a central hypoxic depression cannot fully explain the secondary decline in V_E.

Key words
Ventilatory response • Sustained hypoxia • Carotid body denervation • Rats

Introduction

Ventilatory response to sustained hypoxia typically shows two phases – a rapid, substantial increase in minute ventilation (V_E) followed by its partial decline (roll-off) to a new stable level. Hypoxia-induced increase in central production of inhibitory neuromodulators, such as adenosine (Georgopoulos *et al*. 1989a), GABA (Weil 1994) or endorphins (Kagawa *et al*. 1982), followed by a depression of respiratory neuronal activity was suggested as a possible cause of the ventilatory decline. If this is the case, the decrease in ventilation should correlate with the severity of hypoxia. Moreover, carotid body denervated (CBD) animals (whose ventilation increases during hypoxia only slightly and PO_2 decrease in their CNS should therefore be more pronounced than

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in intact animals) should show at least the same decrease of ventilation during hypoxia as intact animals. Indeed, respiratory depression has been observed in peripherally chemodenervated dogs (Watt *et al.* 1942), goats (Sorensen and Mines 1970) and humans (Holton and Wood 1965). However, others reported that unanesthetized peripherally chemodenervated cats (Miller and Tenney 1975) and dogs (Davenport *et al.* 1947) did not exhibit ventilatory depression during hypoxia. Kimura *et al*. (1998) reported that three patients with bilateral carotid body resection did not show the roll-off during sustained hypoxia. Because the conflicting results could be related to differences in species and methods used, we decided to study the time course of the ventilatory changes during sustained hypoxia in unanesthetized and anesthetized intact rats and carotid body denervated rats.

Methods

The studies were performed on 36 adult male Wistar rats. In the first experiment the response to hypoxia was measured in 8 intact (330±12 g, S.E.M.) and 8 chemodenervated (284±3 g) awake rats, in the second group in 10 intact (342±8 g) and 10 chemodenervated (302±6 g) anesthetized rats.

Carotid body denervation: The experimental rats were anesthetized by halothane inhalation (2 - 2.5 %). The carotid sinus nerves were cut bilaterally from a midline cervical incision.

Measurements: Experiments were done three days after the surgery. Tidal volume (V_T), breathing frequency (f_R) and minute ventilation (V_E) of unanesthetized rats were measured in a plethysmograph using the barometric method described by Bartlett and Tenney (1970). The classic plethysmography (Paleček 1969) was used for the anesthetized rats. The animals were anesthetized with an intraperitoneal injection of pentobarbital (40 mg/kg). The variables were always measured at 5, 10 and 15 min of control air breathing, then at 5, 10 and 15 min of breathing a hypoxic gas mixture (F_IO_2 0.1) and after 5, 10 and 15 min of recovery from hypoxia.

Data analysis and statistics: Each variable was averaged over 5 consecutive respiratory cycles. Results are presented as means ± S.E.M. For statistical analysis, ANOVA with Fisher's PLSD test and Student's t-test were used. Differences were considered significant when $P<0.05$.

Table 1. Minute ventilation (V_E), breathing frequency (f_R) and tidal volume (V_T) in control (C) and chemodenervated (CBD) unanesthetized and anesthetized rats at the 15th min of air breathing, at the 5th and 15th min of breathing hypoxic mixture (10 % O_2 in N_2), and at the 5th min of recovery from hypoxia.

		Unanesthetized rats		**Anesthetized rats**	
		C (n = 8)	**CBD** (n = 8)	**C** (n = 10)	**CBD** (n = 10)
V_E (ml/min)	*air 15´*	188.2 ± 14.5	223.5 ± 23.2	141.8 ± 8.8	136.2 ± 7.5
	hypo 5´	466.2 ± 29.1*	300.2 ± 27.2*$^{\#}$	321.9 ±17.7*	154.9 ± 9.7$^{\#}$
	hypo 15´	389.6 ± 19.3*$^{+}$	324.1 ± 26.1*	278.7 ±16.6*$^{+}$	175.5 ± 18.7*$^{\#}$
	air 5´	171.9 ± 18.9^{+}	178.6 ± 12.6^{+}	140.5 ± 8.3^{+}	142.4 ± 8.1
f_R (c/min)	*air 15´*	90.0 ± 7.2	104.7 ± 6.8	92.6 ± 3.5	113.3 ± 7.7$^{\#}$
	hypo 5´	169.8 ±10.4*	118.2 ± 6.4 $^{\#}$	157.1 ± 8.2*	109.9 ± 7.3$^{\#}$
	hypo 15´	169.0 ± 8.3*	138.0 ± 10.5*	143.5 ± 9.9*	99.2 ± 12.5$^{\#}$
	air 5´	90.6 ± 7.2^{+}	93.1 ± 6.4^{+}	87.0 ± 3.7^{+}	104.2 ± 7.6
V_T (ml)	*air 15´*	2.21 ± 0.3	2.13 ± 0.2	1.54 ± 0.1	1.21 ± 0.0$^{\#}$
	hypo 5´	2.80 ± 0.2	2.54 ± 0.2	2.06 ± 0.1*	1.60 ± 0.1*$^{\#}$
	hypo 15´	2.37 ± 0.2	2.36 ± 0.1	1.99 ± 0.1*	1.70 ± 0.1*
	air 5´	1.91 ± 0.2^{+}	1.95 ± 0.1^{+}	1.61 ± 0.1^{+}	1.45 ± 0.1

**p<0.05 from normoxic values at 15 min, $^{+}$p<0.05 from values at 5 min of hypoxia, $^{\#}$p<0.05 between C and CBD*

Results

All results are summarized in Table 1.

Unanesthetized rats (Fig. 1)

The inhalation of 10 % O_2 for 15 min induced a typical biphasic ventilatory response in unanesthetized controls. The increase in V_E reached 248 % of the control value at 5 min after the onset of hypoxia and this was followed by a partial decline to 207 % of the control value at 15 min of exposure to hypoxia. The increase in ventilation was due to an increase in f_R to 187 % and V_T to 127 % of the control value, respectively.

The control V_E of carotid body denervated (CBD) rats did not differ from those of intact animals, it increased after 5 min of hypoxia to 134 % of the control value and did not decline thereafter, reaching 145 % of control values after 15 min of hypoxia. This increase was realized by a gradual increase in f_R to 133 % of control values, whereas V_T remained stable.

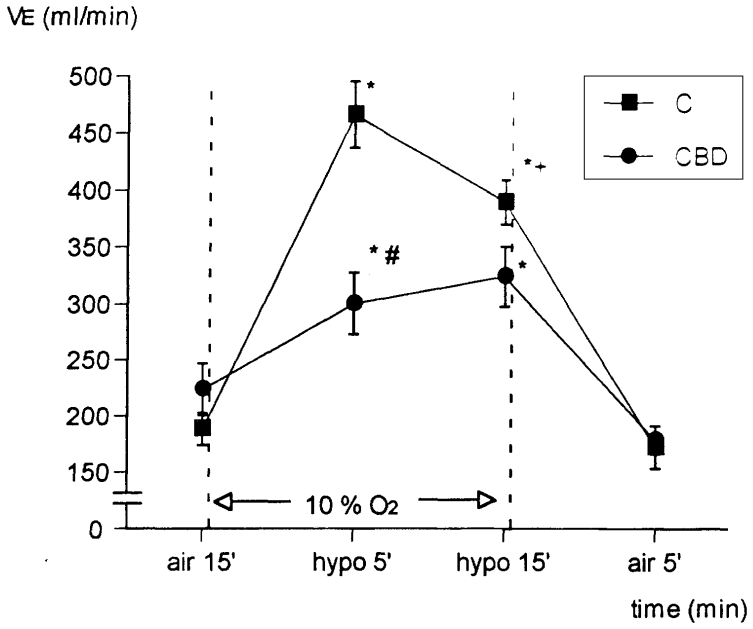

Fig. 1. *Mean values ± S.E.M. of minute ventilation (V_E) in response to hypoxia (10% O_2) in control (C) and chemodenervated (CBD) unanesthetized rats, * $p < 0.05$ from normoxic values at the 15th min, $^{+}p<0.05$ from values at the 5th min of hypoxia, $^{\#}p<0.05$ between C and CBD .*

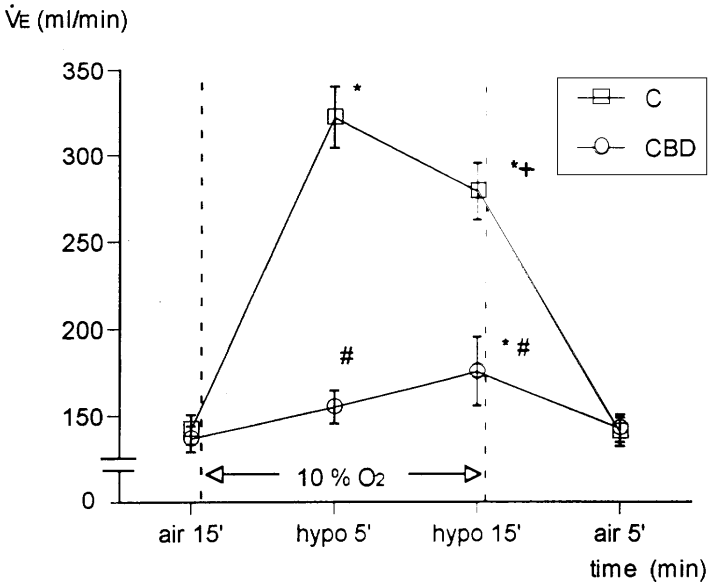

Fig. 2. *Mean values ±S.E.M. of minute ventilation (V_E) in response to hypoxia, (10 % O_2) in control (C) and chemodenervated (CBD) anesthetized rats, * $p < 0.05$ from normoxic values at the 15th min, $^{+}p<0.05$ from values at the 5th min of hypoxia, $^{\#}p<0.05$ between C and CBD.*

Anesthetized rats (Fig. 2)

The V_E of intact anesthetized rats was about half of that of intact awake rats, but the relative change of V_E during hypoxia was very similar (227 % of control ventilation after 5 min of hypoxia). The secondary decrease in ventilation was present (196 % after 15 min of hypoxia). This increase was due to an increase in V_T to 137 % and f_R to 169 % of control values. Control V_E of anesthetized CBD rats was similar to that of anesthetized intact rats. It increased to 114 % after 5 min of hypoxia and did not decrease further during 15 min of hypoxia (129 % in 15 min).

Discussion

The ventilatory response to hypoxia in intact and CBD animals was compared in both anesthetized and unanesthetized animals. The denervation of carotid bodies substantially attenuated, but did not completely block the ventilatory response to hypoxia in our rats. The slightly increased level of ventilation was sustained for 15 min of hypoxia in unanesthetized as well as anesthetized CBD rats without the expected decline. Because the intact rats responded to hypoxia by a large increase in ventilation followed by the typical roll-off, afferentation from carotid bodies and/or the substantial increase in ventilation seems to be crucial for the biphasicity of the response. The fact that CBD rats, which were exposed to the same level of hypoxia as intact animals, did not show the roll-off, suggests that hypoxia-induced depression is not the only cause of the ventilatory decline.

The hypoxia-induced increase of ventilation was in the expected range in both control unanesthetized and anesthetized rats as reported previously (Maxová and Vízek 2001, Vízek and Bonora 1998). The magnitude of the ventilatory response to hypoxia of unanesthetized CBD rats was similar to that reported by Olson *et al.* (1988). The increase of V_E in anesthetized CBD rats, although less pronounced, was surprising since the hypoxic depression was reported as a common response of anesthetized peripherally chemodenervated animals (Neubauer *et al*. 1990). However, rats may depend on other peripheral chemoreceptors (aortic bodies, neuroepithelial cells) more than other species (Martin-Body *et al*. 1985).

As expected, the increase in ventilation in control rats was mainly due to the rate of breathing. V_T changed only in anesthetized controls. In CBD rats, hypoxia accelerated f_R in unanesthetized and augmented V_T in anesthetized animals.

We used poikilocapnic hypoxia since isocapnic conditions would not represent a common response to hypoxia. It is also technically difficult to maintain normocapnia precisely, in particular during the initial increase in ventilation, and even small changes in $PaCO_2$ might markedly influence the response. Although we did not measure $PaCO_2$, the large increase in ventilation in the controls was probably accompanied by hypocapnia, while $PaCO_2$ of experimental rats changed only slightly. A smaller decrease in $PaCO_2$ could result in a disappearance of the roll-off in CBD rats, however, the fact that ventilation also declines during isocapnic hypoxia (Georgopoulos *et al.* 1989b, Long *et al.* 1994) does not support this explanation. It seems that the roll-off also reflects excitatory influences. The initial increase of ventilation in response to hypoxia may result from increased concentration of excitatory neurotransmitter glutamate in CNS resulting from a release of this substance during peripheral chemoreceptor stimulation (Kazemi and Hoop 1991). Central hypoxia may then activate the metabolic pathway converting glutamate to inhibitory transmitter GABA (Kazemi and Hoop 1991), which in turn could cause the secondary ventilatory decline. In this scenario the roll-off cannot appear without substantial initial increase in activity of peripheral chemoreceptors, i.e. in our CBD rats.

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