Roles of Aortic and Carotid Chemoreceptors in Activating the Hypothalamo-Hypophyseal-Adrenocortical System During Hypoxia[1] (36901)

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Previous communications from this laboratory have shown that hypoxia–hypocapnia, induced by breathing 10% oxygen (O_2) at ground level (1–3) or ambient air at simulated altitude of 17,000 ft (4), is a potent stimulus for the activation of the hypothalamo–hypophyseal–adrenocortical (HHA) system. This stressor apparently activates the HHA system via the aortic and carotid bodies since total denervation of these peripheral chemoreceptors prevents the enhanced adrenocortical activity observed in intact hypoxic animals (3, 5, 6). It is well known that stimulation of either the aortic or carotid bodies does not affect the pulmonary and/or cardiovascular systems in the same manner or degree (7–10). Since previous studies concerned with hypoxia and adrenocortical activity were performed on either intact animals (2) or those in which both the aortic and carotid bodies were denervated (3, 5), the present investigation was designed to determine the degree of participation of each pair of chemoreceptors in the HHA reaction to hypoxia. By incorporating into these studies the rates at which Pa_{O_2}, Pa_{CO_2} and pH were altered, an attempt was made to ascertain the degree of hypoxemia–hypocapnia which affects the HHA system.

Materials and Methods. Twenty-eight male mongrel dogs, weighing 14.7 ± 1.8 kg, were anesthetized with sodium pentobarbital (30 mg/kg, intravenously) and maintained at the surgical plane of anesthesia throughout the experiment. The animals were randomly divided into five groups and surgically prepared as follows: (a) intact (sham-operated chemoreceptors) breathing ambient air, (b) intact (sham-operated) breathing 10% O_2 in nitrogen, (c) aortic bodies denervated and breathing 10% O_2, (d) carotid bodies denervated and breathing 10% O_2, and (e) both aortic and carotid bodies denervated breathing 10% O_2.

Tracheotomy was performed to permit the respiring of gas mixtures via a three-way flutter valve connected to a Douglas bag. The right external jugular vein and right femoral artery were cannulated for the infusion of fluids and the collection of anaerobic arterial blood samples, respectively. Respiratory and pulse rates were continuously monitored on an Offner dynograph with a Statham transducer attached to a pneumograph placed around the thorax and another transducer connected to the arterial cannula. The aortic bodies were denervated bilaterally by sectioning the vagi in the cervical region (C_{3-4}), while the carotid bodies were denervated bilaterally at the carotid bifurcation. Immediately following denervation or sham operation of the chemoreceptors, the left lumboadrenal vein (tributaries ligated) was cannulated with polyethylene tubing and a lifting ligature placed around the adrenal vein according to a previously described procedure (4). All animals were allowed to recover for at least 4 hr during which they were breathing 20% O_2 (ambient air) and slowly infused with 0.5 ml saline/min containing 1.0–1.5 mg pentobarbital/ml. Previous studies (2) had shown that adrenocortical secretory rates had attained relatively stable levels at this time and the adrenal cortices were responsive to exogenous adrenocorticotropin (ACTH).

The animal, while still breathing ambient air, was given 2000 IU of heparin and the lumboadrenal vein cannula was opened while placing tension on the lifting ligature. Two

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TABLE I. The Initial Values (Zero Time) of Various Parameters Measured in Intact, Aortic Body (AB) and Carotid Body (CB) Denervated (denerv.) Dogs Prior to the Induction of Hypoxia (10% O_2).

Parameters	Intact		AB denerv.	CB denerv.	AB and CB denerv.
	20% O_2	10% O_2	10% O_2	10% O_2	10% O_2
N[a]	5	6	6	6	5
11-OHCS (μg/min/g)	5.6[b] (2.0)	5.5 (1.2)	6.3 (1.5)	6.0 (1.4)	6.9 (1.2)
Pa_{O_2} (mm Hg)	82.0 (4.9)	80.1 (5.9)	78.7 (6.3)	77.5 (7.3)	76.5 (3.5)
Pa_{CO_2} (mm Hg)	39.4 (4.3)	44.0 (3.4)	38.2 (5.6)	39.7 (3.5)	39.8 (3.0)
pH (units)	7.419 (0.028)	7.378 (0.023)	7.409 (0.025)	7.397 (0.029)	7.390 (0.029)
Resp. (no./min)	18 (5)	16 (3)	12 (3)	15 (4)	13 (3)
Pulse (no./min)	137 (8)	140 (12)	153 (10)	146 (8)	152 (9)

[a] Number of dogs per group.
[b] Mean with standard error given in parentheses.

minutes later adrenal venous blood was collected continuously in graduated test tubes at 1 min intervals for 5 min prior to the induction of hypoxia and for the first 7 min of hypoxia. This was followed by collecting blood at 10, 15, 30, 45 and 60 min during the maintenance of hypoxia. Depending on the rate of lumboadrenal venous blood flow, the cannula was closed after the 10 or 15 min samples to avoid excessive blood loss. A total of seventeen 1 min adrenal vein samples was collected from each dog with the exceptions of those in the combined aortic and carotid bodies denervated group (9 blood samples). Midway during each adrenal sampling period anaerobic femoral arterial blood (about 1 ml) was withdrawn for the determinations of Pa_{O_2}, Pa_{CO_2} and pH with a blood gas analyzer (Instrumentation Laboratory Inc.). Throughout the collection of adrenal venous and femoral arterial blood samples the animals were infused with dextran [6% (w/v) in saline] at a rate which approximated very closely the amount (5–8 ml/min) of blood withdrawn. This was performed to avoid slowly hemorrhaging the animal which is known to cause an increase in peripheral plasma glucocorticoids (11) as well as a monophasic (12) or biphasic (13) response in aldosterone and/or glucocorticoid secretory rates. At the end of the experiment, while still breathing 10% O_2, one or two animals from each group was given 20 U ACTH intravenously to determine if the adrenal cortices had been responding maximally. In all cases exogenous ACTH caused further significant increases in secretory rates.

The blood was centrifuged rapidly at moderate speeds and the plasma was stored at $-25°$ until analyzed for cortisol, the main 11-hydroxycorticosteroid (11-OHCS) of the dog, by either a spectrophotometric (14) or fluorometric (15) method. No consistent or significant differences were noted between the two methods and both methods were used in all groups. At the end of the experiment the cannulated adrenal gland was excised, cleaned and weighed in order to calculate 11-OHCS rates (μg/min/g).

Results. The control values at zero time for 11-OHCS secretory rates, Pa_{O_2}, Pa_{CO_2}, pH, respiratory and pulse rates of all 5 groups are summarized in Table I. Although the respiratory rates were slightly lower and the pulse rates slightly higher in animals with their aortic bodies denervated, perusal of these data reveal no significant differences ($p > .05$) among the various groups for each

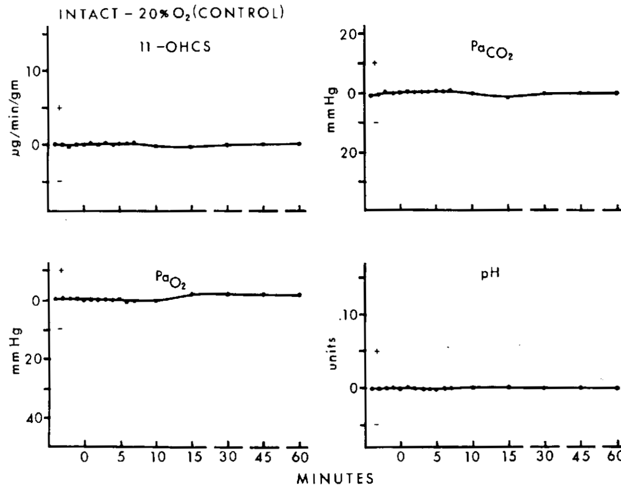

FIG. 1. The mean difference from zero time in 11-OHCS secretory rates, Pa_{CO_2}, Pa_{O_2} and pH of five sham-operated intact dogs breathing ambient air (20% O_2) for 1 hr. F-values for all four parameters: $p > .05$.

parameter measured. Considering that 11-OHCS rates of over 25 µg/min/g can be attained during stressful situations, the initial secretory rates were only moderately elevated 4 hr after surgery and capable of responding to HHA activation. Moreover, analyses of variance of the values obtained for the 5 min preceding the initiation of hypoxia revealed no significant F-values ($p > .05$). The rates of change for each parameter during the first 7 min were 11-OHCS = —0.05 ± 0.07 µg/min, Pa_{O_2} = —0.26 ± 0.18 mm Hg/min, Pa_{CO_2} = —0.02 ± 0.06 mm Hg/min, and pH = —.001 ± .008 units/min.

In general, the intact control animals breathing ambient air for 1 hr showed relatively minor fluctuation from zero time values, and no significant F-values ($p > .05$) in 11-OHCS rates, Pa_{O_2}, Pa_{CO_2} and pH were observed by two way analysis of variance (Fig. 1). The mean differences in respiratory and pulse rates did not vary more than 2 ± 1 breaths/min or 2 ± 3 beats/min, respectively, from control values throughout the entire experiment. In contrast to the control animals, the 11-OHCS rates of the intact hypoxic group (Fig. 2) began to rise significantly ($p < .05$) within the first minute of hypoxia and were approximately 4–5 µg/min/g above prehypoxic rates at 3 min. A small insignificant ($p > .05$) decline occurred at 4 min followed by a rise to maximal rates at 10 min which were maintained for the remainder of the hypoxic period ($p < .01$). The Pa_{O_2} decreased abruptly 15.5 ± 1.5 mm Hg within the first minute of hypoxia and an additional 12.7 mm Hg during the second minute before reaching maximal depressions of 42.5 ± 6.2 mm Hg ($p < .01$) at 7 min, when the respiratory and pulse rates had increased from control levels 23 ± 5 breaths/min and 13 ± 3 beats/min, respectively. On the other hand, the alterations in Pa_{CO_2} and pH were more gradual and attained new steady levels (Pa_{CO_2} = —15.7 ± 1.8 mm Hg and pH = +0.086 ± 0.008) between 7 and 10 min after the induction of hypoxia. The rates at which 11-OHCS, Pa_{O_2}, Pa_{CO_2} and pH were altered during the first 7 min of hypoxia were 0.55 ± 0.12 µg/min, —4.02 ± 1.00 mm Hg/min, —1.30 ± .30 mm Hg/min and 0.011 ± 0.001 units/min, respectively.

Figures 3 and 4 illustrate the effects of denervating either the carotid or aortic bodies on 11-OHCS secretion and arterial blood gases. When only the carotid bodies were denervated (Fig. 3), the 11-OHCS rates began to rise within 1 min after breathing 10% O_2 and attained maximal rates (30–40% of the intact hypoxic group) within 3 min ($p < .05$). These rates were maintained for ap-

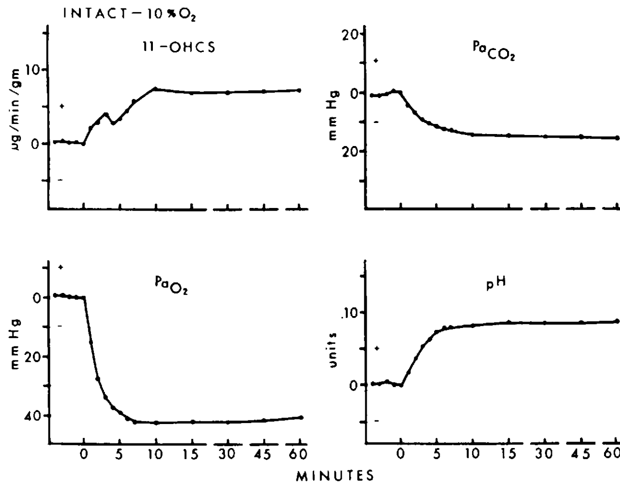

FIG. 2. The mean difference from zero time in 11-OHCS secretory rates, Pa_{CO_2}, Pa_{O_2} and pH of six sham-operated intact dogs breathing 10% O_2 for 1 hr. F-values for all parameters: $p < .05$.

proximately 15 min before gradually declining towards prehypoxic levels. On the other hand, the 11-OHCS secretion of animals with denervated aortic bodies (Fig. 4) required about 7 to 10 min to reach maximal rates (about 35% of the intact hypoxic group) and these rates were maintained for the remainder of the hypoxic period ($p < .05$). The rates of increase in 11-OHCS during the first 3 min were 0.92 ± 0.63 µg/min for the carotid body denervated group, which was essentially similar to the intact hypoxic group (0.99 ± 0.52 µg/min), and 0.54 ± 0.44 µg/min for the aortic body denervated group. The Pa_{O_2} decreased 10.8 ± 2.1 and 12.3 ± 5.0 mm Hg during the first min of hypoxia in the aortic body and carotid body denervated groups, respectively. During the first 7 min of hypoxia, Pa_{O_2} declined at rates of — 4.66 ± 1.03 mm Hg/min for the former group and — 4.64 ± 0.97 mm Hg/min for the latter group. These rates were not significantly ($p > .05$) different from each other nor from the intact hypoxic group. However, the declines in Pa_{CO_2} and the concurrent elevations in arterial pH of individually denervated

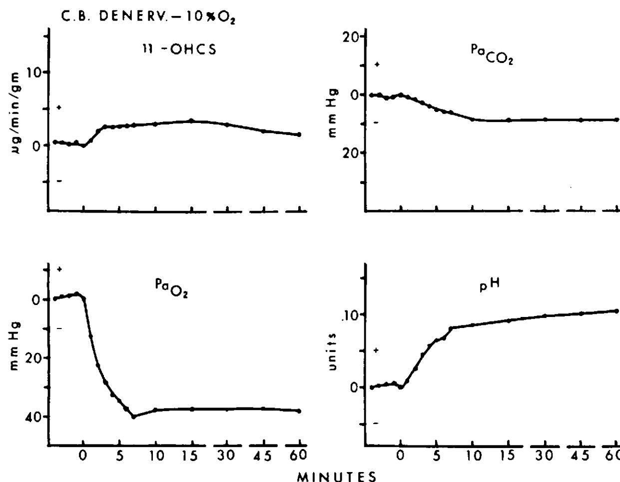

FIG. 3. The mean differences from zero time in 11-OHCS secretory rates, Pa_{CO_2}, Pa_{O_2} and pH of six dogs with denervated (Denerv.) carotid bodies (CB) breathing 10% O_2 for 1 hr. F-values for all four parameters: $p < .05$.

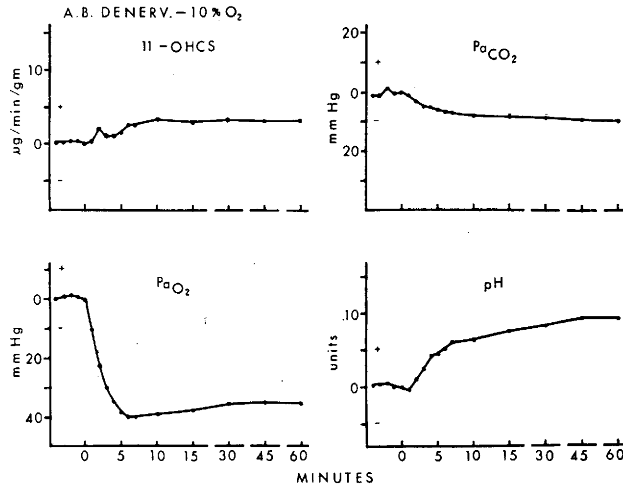

FIG. 4. The mean differences from zero time in 11-OHCS secretory rates, Pa_{CO_2}, Pa_{O_2} and pH of six dogs with denervated (Denerv.) aortic bodies (AB) breathing 10% O_2 for 1 hr. *F*-values for all four parameters: $p < .05$.

groups took approximately 2 to 5 min longer to reach new steady levels than those observed in the intact hypoxic group. This phenomenon was also evident in the slower rates of rise in respiratory and pulse rates of the animals with denervated peripheral chemoreceptors. Thus the rates at which Pa_{CO_2} and pH changed during the first 7 min of hypoxia were — 0.91 ± 0.28 mm Hg/min and 0.010 ± 0.002 units/min for the aortic body denervated group, and — 1.02 ± 0.15 mm Hg/min and 0.011 ± 0.002 Units/min for the carotid body denervated group.

Unlike the intact animals or those with only one pair of denervated chemoreceptors, the induction of hypoxia in dogs with both aortic and carotid chemoreceptors denervated (Fig. 5) resulted in no significant ($p > .05$) changes in 11-OHCS secretion throughout the experimental period; however, the Pa_{O_2} of these animals was depressed (— 31.5 ± 3.1 mm Hg at 5 min) as much as that observed in the intact hypoxic group, while the changes in Pa_{CO_2} and pH, though significant ($p <$

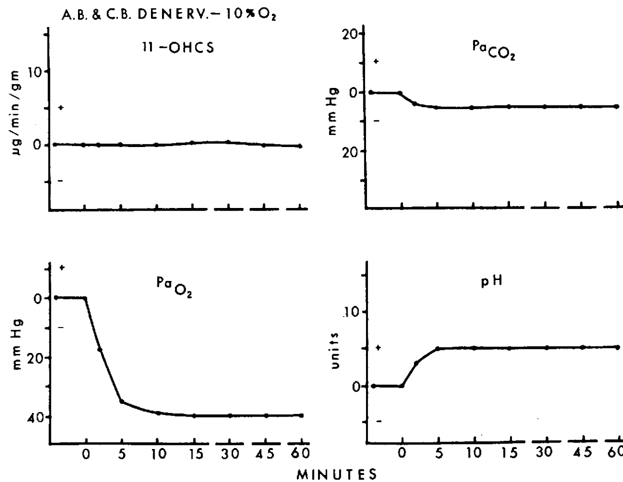

FIG. 5. The mean difference from zero time in 11-OHCS secretory rates, Pa_{CO_2}, Pa_{O_2} and pH of five dogs with denervated (Denerv.) aortic (AB) and carotid (CB) bodies breathing 10% O_2 for 1 hr F-values for Pa_{CO_2}, Pa_{O_2} and pH: $p < .05$; while for 11-OHCS: $p > .05$.

.05), were slower to develop and less marked. The respiratory and pulse rates tended to rise but not significantly ($p > .05$).

Discussion. The intense activation of the HHA system of intact hypoxic dogs, as measured by 11-OHCS secretory rates, was partially inhibited by either denervation of the aortic or carotid bodies and completely abolished by denervation of both sets of peripheral chemoreceptors. The latter observation is in accord with previous findings (3, 5) and indicates that hypoxemia does not act directly on the adrenal cortex. The abrupt drop in Pa_{O_2} noted during the first minute of hypoxia undoubtedly was sufficient to increase the number of afferent impulses from the chemoreceptors and thus account for the rapidity with which the HHA system was activated. The difference in rates at which 11-OHCS secretion increased during the first few minutes of hypoxia in animals with only functioning aortic bodies (0.92 μg/min) and those with only functioning carotid bodies (0.54 μg/min) suggests that the initial rapid rise in 11-OHCS rates noted in intact hypoxic dogs may be due mainly to stimulaton of the aortic chemoreceptors. On the other hand, the tendency for 11-OHCS rates to gradually decline 15–30 min following the induction of hypoxia in the carotid body denervated animals, but not in the aortic body denervated dogs, suggests that stimulation of the carotid bodies is necessary for the maintenance of the elevated adrenocortical secretory rates noted during hypoxia. This difference in aortic and carotid chemoreceptor responses may be due to their difference in sensitivity to and/or interactions of Pa_{O_2} and Pa_{CO_2} at the receptor level. Thus it is conceivable that the activation of the HHA system by hypoxia is composed of a fast component (rapid rise in 11-OHCS) which is triggered mainly by stimulation of the aortic bodies, and a maintenance component which involves primarily continual stimulation of the carotid bodies; however, in order to obtain rapid and maximal increases in 11-OHCS rates during hypoxia both peripheral chemoreceptors must be functioning.

In 1939, Comroe (7) clearly showed that acute hypoxemic stimulation of the carotid body of dogs affects the respiratory center more than the vasomotor center, whereas the aortic body exhibits the opposite effect. In general, the cardiopulmonary responses as indicated by changes in respiratory and pulse rates of the hypoxic dogs used herein followed the above patterns; however, the 11-OHCS rates indicate that the aortic and carotid bodies equally contribute approximately 30–40% of the adrenocortical response observed in intact hypoxic animals. It is puzzling at this time to ascertain why the arithmetic summation of these two responses is less than that noted in intact hypoxic dogs. It is obvious that the afferent neural discharges from the chemoreceptors, which reach the hypothalamo–hypophyseal complex for the release of ACTH, elicit responses in this complex which are different from those arriving at the respiratory and vasomotor centers. Although all the afferent neural projections from the aortic and carotid bodies which enter the brain stem have not been elucidated completely, a mechanism similar to that observed for the interaction of baroreceptor afferent impulses from the aortic and carotid baroreceptors at the nucleus tractus solitarii may also exist for chemoreceptor afferents (16). Thus, one could postulate an interaction of neural impulses from the peripheral chemoreceptors in some neuronal pool within the complex resulting in 11-OHCS rates higher than can be accounted for by the arithmetic summation of impulses from both sets of chemoreceptors.

It is doubtful that the absence of a rapid 11-OHCS response in the aortic body denervated group was due to the effects of bilateral vagotomy (elimination of Hering-Breuer reflex, *etc.*) on pulmonary ventilation, since the rates of change in Pa_{O_2}, Pa_{CO_2} and pH were essentially similar in both the aortic body denervated and carotid body denervated groups made hypoxic 4 hr after denervation. However, it has been shown that 10 min after bilateral cervical vagotomy of anesthetized dogs there is a prompt rise in 11-OHCS rates followed by a return to control levels within 2 hr; and if at this time the carotid arteries are bilaterally constricted, maximal 11-OHCS secretion is observed

(17). It has been proposed that impulses arising from intrathoracic vascular receptors and transmitted via the vagi tonically inhibit the release of ACTH (18). Although the vagi undoubtedly play an important role in potentiating the adrenocortical response to constriction of the carotid arteries, it is obvious from the results presented herein that bilateral vagotomy does not potentiate the response of the HHA system when hypoxia is the stimulus, otherwise higher 11-OHCS rates would have been noted in the aortic body denervated group than in the carotid body denervated or intact groups. Likewise it is doubtful that the baroreceptor mechanism involved in hypotension or hemorrhage plays a major role in the activation of the HHA system during hypoxia, since it is well known that hypoxemia reflexly causes vasoconstriction resulting in a rise in arterial pressure which would in all probability prevent this mechanism from increasing adrenocortical activity. Furthermore, the frequency of neural discharges from the carotid bodies decreases with increasing pressure within the carotid sinus, while the frequency of discharges increases with decreasing Pa_{O_2}, and the latter is not affected by simultaneous elevations of arterial pressure (19, 20). If a similar phenomenon can be attributed to the aortic bodies, then it is reasonable to assume that the baroreceptors do not play a major role in the hypoxic activation of the HHA system.

Many of the cardiopulmonary reflexes that affect adrenocortical secretory rates also influence the release of the antidiuretic hormone (ADH). This hormone has been implicated in the activation of the HHA system in some stresses, since it can cause the release of the corticotropin-releasing factor (CRF) stored in neurons of the median eminence of the hypothalamus (21), as well as potentiate the action of CRF on the adenohypophysis for the release of ACTH and possibly act directly on the adrenal cortex (22). It is well known that although the occlusion of both common carotid arteries results in the elevation of peripheral plasma levels of ADH (23), perfusion of the isolated carotid bodies (intact sinus nerve) with completely deoxygenated normocapnic blood causes elevations in plasma titers of ADH in bilaterally vagotomized dogs but not in animals with intact vagi (24). Thus, the stimulation of ADH release by the chemoreceptors is a relatively weak reflex in intact animals. Furthermore, Currie and Ullmann (25) demonstrated that hypoxia, as well as other respiratory maneuvers which affect intrathoracic pressure, increase left atrial receptor activity and consequently inhibit the release of ADH resulting in polyguria. Thus, it is highly improbable that ADH plays an important role in the stimulation of the HHA system during hypoxia.

Summary. Anesthetized mongrel dogs with cannulas in the left lumboadrenal vein were subjected to hypoxia in order to determine the participation of the aortic and carotid bodies, separately and combined, in the adrenocortical response to this stressor. In intact dogs the induction of hypoxia by breathing 10% O_2 at ground level for 1 hr caused rapid and sustained maximal 11-OHCS rates which paralleled the alterations in arterial oxygen, carbon dioxide and pH. On the other hand, only 30–40% of these maximal 11-OHCS rates were observed in hypoxic animals with either denervated aortic or carotid chemoreceptors. The adrenocortical response to hypoxia was abolished by denervating both aortic and carotid chemoreceptors while essentially similar changes in arterial gases and pH as those observed in intact hypoxic dogs were seen. These data demonstrate not only the necessity of functioning peripheral arterial chemoreceptors for maximal response of the hypothalamo–hypophyseal–adrenocortical system to hypoxia, but also that the aortic and carotid chemoreceptors participate equally in this response.

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