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Vagal Control of Heart Rate during Hypoxia in the Cat[1] (38385)

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Peripheral receptors that respond to altered O_2 tension in the blood have been associated with the aortic and carotid bodies in mammals (1). The classical cardiorespiratory adjustments which result from stimulation of these receptors with hypoxia are increased respiratory minute volume (2), increased mean arterial pressure (secondary to peripheral vasoconstriction) (3–5), and bradycardia (predominantly vagal in origin) (6). The first two responses are readily reproducible, even in the anesthetized preparation, and occur at absolute thresholds of PaO_2 of 65–75 mm Hg (7). Conversely, the bradycardia of hypoxia varies considerably with the species, the respiratory pattern of the hypoxic animal, and the type of anesthesia used (8–12).

Diving mammals, for example, exhibit a marked bradycardia in response to hypoxia which is vagal in origin and is virtually abolished by chemoreceptor denervation (13). In contrast, freely breathing, awake terrestrial mammals often show an initial tachycardia consequent to hypoxia, with bradycardia appearing only in late or severe hypoxia (PaO_2 less than 40 mm Hg) (5, 9, 14). Alternatively, when ventilation is controlled in the same preparation, bradycardia occurs without an initial tachycardia (5, 8–11, 14).

In order to explain the apparent paradoxes and unpredictability in the response of heart rate to hypoxia, it has been proposed that various other vagally mediated reflexes, elicited either directly or indirectly by the hypoxic episode, interact with the vagal component of the chemoreceptor reflex to ultimately determine heart rate (6). Daly and co-workers (15) have presented good evidence that in the dog a "lung inflation" reflex may interact with the chemoreceptor reflex in such a manner as to produce virtually any heart rate change, depending on the level of hypoxia and the extent of the respiratory response.

Before such hypotheses can be evaluated, as has previously been stressed (6), it is necessary to determine the nature of heart rate control by the peripheral chemoreceptors in the absence of other complicating factors. In the following experiments we have used the awake spinal cat (encéphale isolé) to study the bradycardia of hypoxia. In this way the effects of anesthesia, which are predominantly vagolytic (12), respiratory changes, and blood pressure variations can be controlled or eliminated. Accordingly we have been able to delineate certain features of the "primary" vagal component of the isolated chemoreceptor reflex. In particular we have addressed ourselves to the following questions: (a) is there a well-defined threshold of PaO_2 for the appearance of a chemoreceptor-mediated vagal bradycardia?; (b) what are the relative contributions to the observed bradycardia of the aortic and carotid chemoreceptors?; (c) is there a vagal bradycardia which is due to the direct effects of hypoxia on the central nervous system?

Materials and Methods. Preparation. Twelve male cats weighing between 3.0 and 4.0 kg were anesthetized with halothane, tracheotomized, and ventilated mechanically with a Harvard respirator. The cats were placed in a stereotaxic instrument, the spinal cord was exposed at the level C_1–C_2, and a transection was performed using suction and crushing. The animals were then placed in a supine position and inhalation anesthesia was discontinued. A laparotomy was performed and a rubber tube was introduced into the stomach through its anterior wall to avoid bloating from swallowed air and the possible

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initiation of reflexes related to gastric distention. After all surgical procedures, wounds were sutured and 2% lidocaine was applied to the wound margins.

Four male cats weighing between 3.0 and 4.0 kg were anesthetized with sodium pentobarbital, 30 mg/kg ip. The animals were laid supine, tracheotomized, and ventilated as above. In addition a bilateral pneumothorax was performed in order to dissociate respiratory movements from lung inflation, and a resistance of 3 cm H_2O was placed in series with the outflow tube from the pump.

In each of the 16 cats, the thoracic aorta and inferior vena cava were cannulated via the right femoral artery and vein, respectively, to allow withdrawal of blood or injection. Cannulae introduced through the left femoral artery and vein were attached to Statham P23AC pressure transducers for monitoring of aortic and intrathoracic caval pressures.

In the 12 spinal cats systemic arterial pressure, mean arterial pressure, central venous pressure, and heart rate were simultaneously recorded on a Grass Model 7 polygraph. Heart rate was computed electronically by a Grass tachograph triggered by the arterial pressure wave, and tracheal pressure was measured via an intratracheal catheter attached to a Statham P23AA pressure transducer. In the anesthetized cats, in addition to the above, chest movements were monitored via a pneumograph attached to a pressure transducer.

End-tidal CO_2 was periodically measured with a Beckman LB-2 Medical Gas Analyzer, and arterial PO_2, PCO_2, and pH were determined from aortic blood samples by a Radiometer Blood Microsystem.

Testing. The spinal cats were allowed to recover completely from the anesthesia (2 hr) and then given 2 mg iv propranolol (sufficient to block heart rate responses to 2 μg iv isoproterenol), in order to abolish all adrenergic influences on heart rate. Hypoxia was induced by administering various predetermined mixtures of O_2 and N_2, with or without CO_2, for periods of 1–30 breaths. Alternatively, apneic asphyxia was induced by opening the tracheal connection to the pump. At appropriate times arterial blood samples were withdrawn to follow the time course of the hypoxia.

Progressive denervation was performed as follows: in 4 of the 12 spinal cats, the two sinus nerves were first transected. Two of these then had a bilateral aortic nerve transection at the junction with the superior laryngeal nerve. In four cats, the aortic nerves were transected first; two then had a sinus denervation performed. Two cats had both sets of nerves transected simultaneously and the remaining two cats were vagotomized. Before each nerve section the cats were reanesthetized, and afterward allowed 2 hr recovery time before retesting. For the final series of tests the 10 nonvagotomized animals were given 0.1 mg/kg atropine. Throughout the course of the experiments, β-blockade was maintained with propranolol and mean arterial pressure was maintained at 75 ± 5 mm Hg by venous bleeding or iv norepinephrine infusion.

The testing procedures were similar in the cats anesthetized with barbiturate with the exception that β-blockade was not maintained. Of the four initial cats, two had a sinus denervation followed by aortic denervation and two were denervated in the opposite order. All four cats were vagotomized for the final series of tests.

Results. Experiments in unanesthetized spinal cats. 1. When hypoxia was induced by stopping the pump, and thus removing all respiratory movements, a bradycardia appeared consistently. The first signs of heart rate slowing, defined as a decrease of 5–10 beats/min below minimum resting normoxic levels, occurred with an average latency of 19.8 ± 1.3 sec in 22 separate trials (Table I). If apnea was allowed to continue beyond the threshold, a very marked bradycardia rapidly ensued, often leading to cardiac arrest. At this time the PaO_2 was generally 40–50 mm Hg and the $PaCO_2$ 33–38 mm Hg.

TABLE I. Latency of Bradycardia in Response to Apneic Asphyxia.

Status	Number of trials	Time (sec)[a]
Sinus and aortic nerves intact	22	19.8 ± 1.3
Aortic denervation	9	18.0 ± 2.4
Sinus denervation	7	72.8 ± 1.8[b]
Sinoaortic denervation	10	74.7 ± 2.0
Atropine or vagotomy	10	>120[c]

[a] All values are mean ± standard error.

[b] The difference between this and the previous entry is significant at the $P < 0.01$ level by the Student–Newmann–Kuels test for unequal sample sizes (23).

[c] This difference is significant at the $P < 0.05$ level.

2. The next series of experiments elaborated the relation between the PaO_2 and $PaCO_2$ at the start of bradycardia, as defined above. Over the range of $PaCO_2$ studied (20–30 mm Hg) the threshold PaO_2 was found to vary linearly with $PaCO_2$ (Fig. 1). The equation defining the least-squares regression line for the 20 data points (10 cats) collected is given by $PaO_2 = 1.29\,PaCO_2 + 34$.

3. We next tried to determine whether there was any significant difference in the time course of bradycardia between the responses to hypoxia of the aortic and the carotid chemoreceptors. The "carotid cat" (aortic denervation) had a latency to bradycardia of 18 ± 2.4 sec (nine trials) while the "aortic cat" (sinus denervation) had a significantly different lag of 72.8 ± 1.8 sec (seven trials) before bradycardia appeared. The delay before bradycardia developed in the "carotid cat" did not differ significantly from that in the intact animal (Table I). Similarly, the "aortic cat" showed a delay that did not differ significantly from the sino-aortic denervated animal: 74.7 ± 2.0 sec (10 trials). Figure 2 shows typical records of bradycardia in response to apnea in cats with aortic and sinus denervation.

4. After atropine or vagotomy the latent period for bradycardia increased to more than 120 sec for 10 trials (Table I). At this time, respiration was resumed in order to prevent hypoxic damage to the animal.

5. To further distinguish between the cardiac vagal effects of carotid and aortic chemoreceptor stimulation, the two-breath test with 100% N_2 was used. The effect of two breaths of 100% N_2 was to send a pulse of hypoxic blood of 8- to 12-sec duration, with a minimum PaO_2 of about 45 mm Hg, through the system. Typical responses of the "carotid" and "aortic" cats are shown in Fig. 3. The "carotid cat" responded with a transient bradycardia to the hypoxic pulse while the "aortic cat" showed no bradycardia.

Experiments in pentobarbital-anesthetized cats. In order to compare the extent of vagal control by the two chemoreceptive areas with other cardiovascular and respiratory responses, the pulse test was performed in cats under barbiturate anesthesia. (Heart rate responses of short latency are usually abolished by the barbiturate, but vascular and respiratory responses remain intact.) Both "aortic" and "carotid" cats under barbiturate anesthesia consistently showed a transient increase in systemic arterial pressure (10-15 mm Hg) after receiving a two-breath pulse of N_2 (Fig. 4, upper tracings); how-

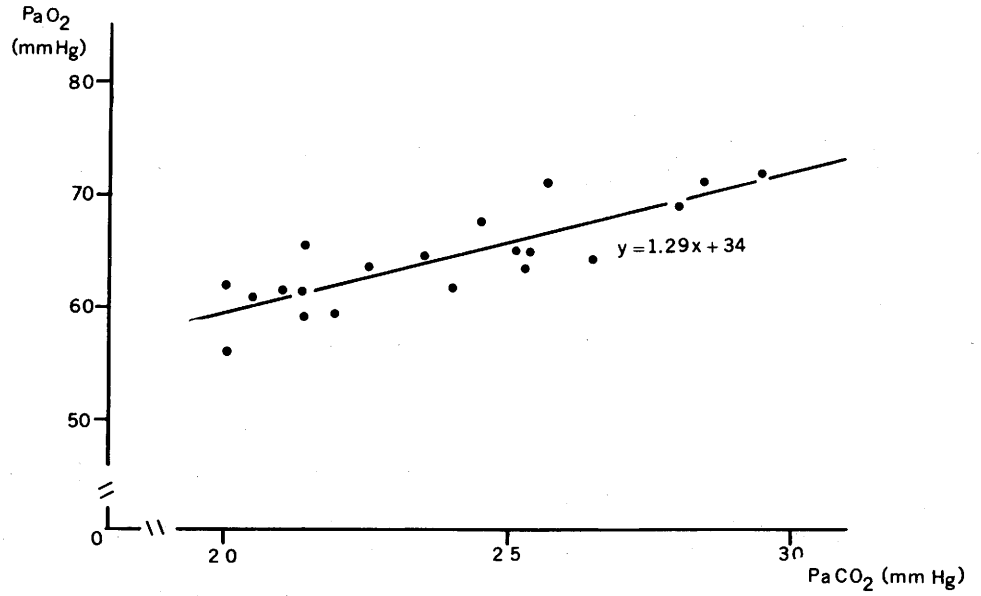

FIG. 1. Threshold for the appearance of bradycardia in response to hypoxia. Spinal cats were ventilated with various mixtures of O_2, CO_2, and N_2. At the appearance of bradycardia an arterial blood sample was drawn and PaO_2 and $PaCO_2$ were measured. Each point represents a single test. The straight line is the least-squares regression for the 20 data points from 10 cats.

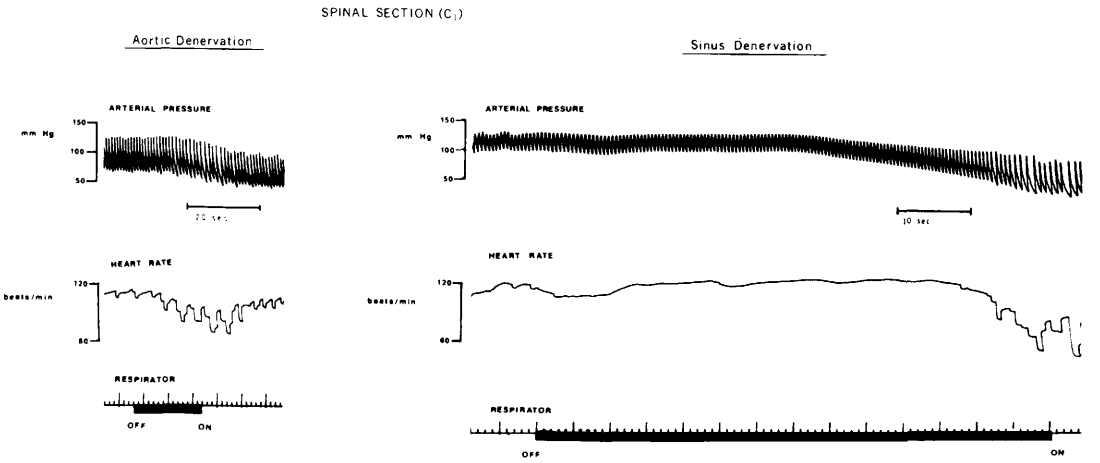

FIG. 2. Bradycardia in response to apneic asphyxia. Apneic asphyxia was induced by opening the tracheal connection to the respirator in spinal cats with section of either both aortic nerves (record on left) or both sinus nerves (record on right). Note absence of bradycardia after sinus denervation.

ever, only the "carotid" cat responded with a two- to four-breath augmentation of ventilatory movements (20–30% above the resting levels). The "aortic" cat showed no change in chest movements after a N_2 pulse. The time lag for the appearance of reflex changes in all three cases was 9–13 sec, about the same as for bradycardia in the spinal cat.

Discussion. A. In our experiments we took advantage of the fact that in the spinal cat (C_1-section), or encéphale isolé, all spontaneous respiratory movements are absent, even when the PaO_2 reaches fatal levels, and could thus completely control lung inflation during hypoxic episodes without autonomous interference by the cat. A bradycardia in response to hypoxia

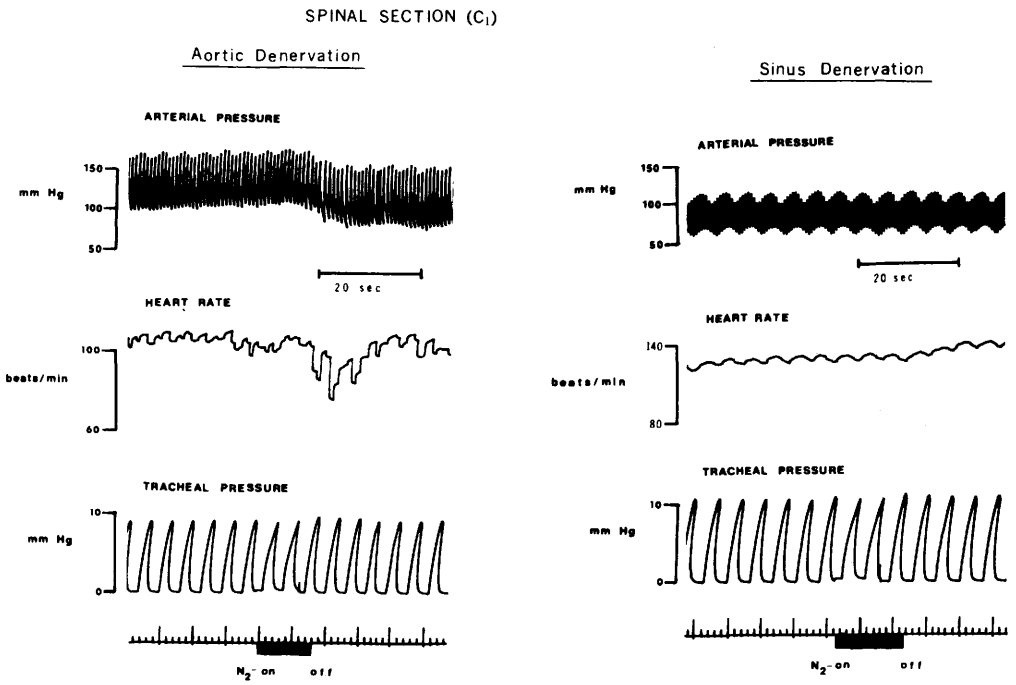

FIG. 3. Bradycardia in response to 100% nitrogen. Spinal cats with either aortic nerve or sinus nerve section were artificially ventilated with 100% N_2 for two breaths. Note absence of bradycardia after sinus denervation.

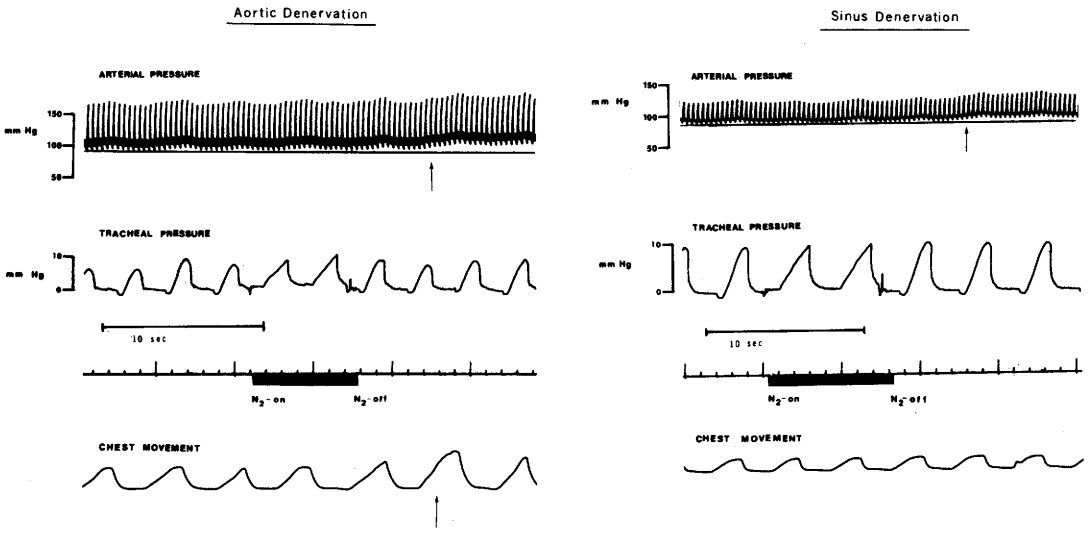

FIG. 4. Pressor and respiratory responses to 100% nitrogen. Cats anesthetized with 30 mg/kg pentobarbital ip were artificially ventilated for two breaths with 100% N_2 after section of aortic nerves (left) or sinus nerves (right). Note that pressor responses occurred in cats with either an aortic or a sinus denervation (upper tracings), while a respiratory response occurred only in cats with sinus nerves intact, *i.e.*, aortic denervation (lower tracings). Arrows indicate onsets of responses.

was consistently observed in all cats whether the animal was allowed to become apneic in expiration by opening the tracheal connection to the respirator (no lung movements), or the tidal and minute volumes were kept constant by mechanical ventilation while hypoxic gas mixtures were administered. Thus, in the absence of lung inflation and other respiratory adjustments to hypoxia the primary reflex response of the peripheral chemoreceptors includes vagal bradycardia, in agreement with the findings of other workers who have attempted to control ventilatory movement during hypoxia (6).

B. Although there is now a growing accord that bradycardia is, in fact, a primary response of the chemoreceptors, the nature of the experimental designs of previous investigators has not permitted the measurement of thresholds for its appearance. In our experiments bradycardia appeared at well-defined levels of PaO_2 which varied linearly over the experimental range of $PaCO_2$ used. This interesting variation in the threshold for bradycardia with $PaCO_2$ may result from the singular behavior of chemoreceptor afferent discharge during hypoxia as deduced from single-unit recording. Whereas single-unit discharge in the normoxic animal remains relatively constant for variations of $PaCO_2$ from 20–40 mm Hg, in the hypoxic animal, small increases in $PaCO_2$ markedly increase discharge rates at constant levels of PaO_2 (16). Numerous explanations of this phenomenon have been proposed (17), but to date none has received general acceptance. Our data indirectly support the proposition that chemoreceptor sensitivity to O_2 is a function of $PaCO_2$, but we can draw no conclusions about possible receptor mechanisms.

The absolute level of PaO_2 at which bradycardia appeared in the normocapnic animal ($PaCO_2$ = 26–28 mm Hg) (18) was 70 ± 5 mm Hg. This is significant because it correlates with data on thresholds for the appearance of pressor and respiratory effects (7). As a result, we may expand our conclusions: not only is vagal bradycardia an intrinsic component of the integrated response of the peripheral chemoreceptors to hypoxia, but it occurs with the same thresholds and latencies as the other components, and thus inconsistencies in its appearance must be attributable to secondary or overriding factors rather than to inherent variations in the response itself.

C. A divergence between the aortic and carotid chemoreceptors in the control of respiration has been suggested by clinical observations in man when carotid denervation has been performed as a treatment for asthma. In these instances a marked reduction in respiratory responsiveness to hypoxia has been observed (19). Comroe *et al.*, using injection techniques in the

dog, reported a difference in reflex heart rate responses between the two areas (20, 21), but to date no conclusive data on vagal control by the aortic chemoreceptors have been published.

From our experiments it appears that the carotid body accounts for the predominant if not the complete reflex vagal response of the peripheral chemoreceptors to hypoxia. The "carotid cat" responded with bradycardia after latencies which closely approximate those of the intact cat. The "aortic cat," on the other hand, showed a greatly prolonged time course for the appearance of bradycardia which did not differ significantly from that in sinoaortic denervated animals, where only central effects remained intact.

The two-breath pulse test with 100% N_2 yielded results which suggest even more striking conclusions. The failure of the spinal "aortic cat" to respond with bradycardia to the N_2 pulse indicates that the aortic chemoreceptors have no significant vagal representation as part of their reflex profile. Our experiments with cats under barbiturate anesthesia may indicate that the aortic body also has no significant input for the control of respiration.

The implication of all this is that the carotid body alone is responsible for the vagal and respiratory components of the integrated response to hypoxia, but that both the aortic and carotid chemoreceptors participate in the pressor response. From this it is tempting to postulate that the various components of the chemoreceptor reflex may be integrated at different levels in the brain stem and that the vagal component may more closely resemble the respiratory component in having a significant supramedullary representation (22).

D. Lastly, our experiments in the spinal cat clearly demonstrate a central response to hypoxia containing a component of vagal bradycardia. The mechanism of this central bradycardia remains unclear. Whether there is a central chemoreceptor area sensitive to O_2 or whether the bradycardia results from the direct effects of hypoxia on the vagal motor pool itself cannot be deduced from our experiments. We can state positively, however, that a vagal bradycardia of central origin is observed significantly earlier than the minimum of 120 sec before the direct effects of hypoxia on cardiac metabolism begin to exert a slowing effect on the heart.

Summary. In spinal cats whose ventilation is completely controlled the primary cardiac response to hypoxia includes vagal bradycardia. This bradycardia appears at distinct levels of PaO_2 which vary as a function of $PaCO_2$. The carotid chemoreceptors are chiefly responsible for eliciting reflex bradycardia while the aortic chemoreceptors make little or no contribution. This divergence of function is mirrored in the reflex control of respiration but not in the pressor response to hypoxia where both sets of receptors are important. A bradycardia which results directly from the action of hypoxia on the central nervous system can be distinguished from both peripheral reflex effects and the direct effects of hypoxia on the heart itself.

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