Effect of Hypercapnic Acidosis and of Hypoxia on Adrenal Catecholamine Output of the Spinal Dog.* (31154)

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Acute and severe elevation of pCO_2(11) and decrease in pO_2(5) have been associated with sympatho-adrenal stimulation and increased plasma catecholamine levels in the dog. Increased pCO_2 appears to be a more potent stimulus, but in some instances such as asphyxia a decrease in pO_2 is also present and it is difficult to assess the relative importance of each. It was assumed by some investigators that these stimuli act on the central and peripheral chemoreceptors and that "except in very high concentrations, the locus of action of CO_2 does not seem to be a direct one on the adrenal medulla"(16). More recently, Morris and Millar(8) have reported "that below about pH 6.8 a direct effect of CO_2 on the adrenal medullary cells is partly responsible for catecholamine liberation."

The purpose of this study was to investigate selectively the effects of low pO_2 and high pCO_2 on adrenal catecholamine output in the spinal dog. An attempt was also made to determine whether the effect of an elevated pCO_2 could be attributed to increased $[H^+]$.

Methods. Mongrel dogs weighing 9.1-19.8 kg were anesthetized with sodium pentobarbital (25 mg/kg), and were intubated and mechanically ventilated with room air. Both femoral arteries were cannulated and a catheter was inserted into the right lumbo-adrenal vein as described by Hume and Nelson(4). The spinal cord was completely transected at the level of the eighth cervical segment (C_8).

Ventilation was initially adjusted to assure normocapnia and after one or two hours control measurements were made. The dogs were then ventilated with a series of different gas mixtures for a period of 15 minutes each in the following manner: 8% O_2 and 92% N_2; 10% CO_2, 25% O_2 and 65% N_2; or 20% CO_2, 30% O_2 and 50% N_2; and air.

Adrenal venous samples were taken periodically for catecholamine determinations and at the end of each adrenal collection an arterial blood sample was taken for measurement of pH, pCO_2, O_2 saturation and glucose. In several dogs, these measurements were also made on adrenal venous blood. Blood pressure was monitored continuously with a Statham strain gauge and a Grass Model 5 recorder. Body temperature was recorded with a thermistor probe placed in the esophagus and was maintained about 35°C by means of a heating pad. Epinephrine and norepinephrine concentrations in the adrenal venous blood were determined by the Manger modification(5,13) of the ethylenediamine fluorometric method of Weil-Malherbe and Bone(19). Adrenal catechalomine output (ACO) was calculated (millimicrograms/kg/min) by multiplying the total concentration of epinephrine and norepinephrine in the adrenal plasma by the adrenal plasma flow during the collection. Acid-base measurements were determined with the Astrup microequipment and the Siggaard-Andersen nomogram(17). Blood glucose was determined by the glucose oxidase method(15). Oxygen saturation was measured by the technique of Nahas(9) and pO_2 was calculated from the O_2 dissociation curve.

Results. The severe acidosis (mean pH 6.93) induced by the inhalation of 10-20% CO_2 was accompanied by a consistent increase in adrenal catecholamine output which was predominantly due to a rise in epinephrine concentration (Table I). This increase (mean 1.5-12.3 mμg/kg/min) was related to the severity of the acidosis. ACO was in all instances only slightly elevated when the pH did not fall below 7.0. ACO returned almost

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TABLE I. Effect of Hypercapnic Acidosis on ACO (Mean Values 6 Animals).

	pH	$PaCO_2$ (mm Hg)	BP (mm Hg)	BG*	ACO†
#1 Normocapnia	7.42	26	101	83	1.5
#2 Hypercapnia	6.93	127	62	97	12.3
#3 Normocapnia	7.44	30	119	83	2.2

* BG—blood glucose in mg/100 ml.
† ACO—mean adrenal catecholamine output in mμg/kg/min.

Analysis of variance performed on the logarithms of the individual ACO values indicates that the mean of logarithms of #2 is significantly higher than #1 and #3 ($p < 0.01$), and that #1 does not differ significantly from #3.

TABLE II. Effect of THAM Correction of Hypercapnic Acidosis on ACO (Mean Values 3 Animals).

	pH	$PaCO_2$ (mm Hg)	BP (mm Hg)	BG*	ACO†
#1 Normocapnia	7.41	28	90	97	.6
#2 Hypercapnia	6.96	122	62	123	2.5
#3 Hypercapnia and THAM	7.38	88	97	77	1.4

* BG—blood glucose in mg/100 ml.
† ACO—mean adrenal catecholamine output in mμg/kg/min.

Analysis of variance performed on the logarithms of the individual ACO values indicates that the mean of the logarithms of #2 is significantly higher than #1 and #3 ($p < 0.01$).

to control levels (2.2 mμg/kg/min) 15 minutes after ventilation with room air was resumed. When THAM was administered (50-100 ml of a 0.3 M solution) during hypercapnia (pCO_2 88 mm Hg) and acidosis was corrected (pH 7.38), an abrupt decrease in ACO occurred in all instances (Table II).

Acute hypoxia (PaO_2 36 mm Hg) in the spinal dog was not associated with an increase in ACO (Table III) when no concurrent appreciable acidosis developed.

There were similar changes in adrenal venous and peripheral arterial pH, pO_2, pCO_2 and base excess during the 3 experimental periods. Blood glucose levels increased from an average of 83 to 97 mg/100 ml during the hypercapnic periods (Table I), but did not increase during hypoxia (Table III). When THAM was administered, glucose concentration fell from an average of 123 to 77 mg/100 ml (Table II).

Discussion. This study indicates that in the spinal dog, lowered pO_2 (mean, 36 mm Hg), when not accompanied by acidosis, has no appreciable stimulating effect on adrenal catecholamine output. However, hypercapnia is a potent stimulus to catecholamine liberation in the C_8 spinal dog. After severing all nerve connections between the adrenal gland and the brain by section of the spinal cord at C_8, hypercapnic acidosis still produced an increase in adrenal medullary secretion. Our findings differ from those reported by Tenney (16) that hypercapnia in pithed cats does not elicit any sympatho-adrenal response and that "spinal dogs never gave evidence of sympatho-adrenal activation under CO_2 stress." It should be noted that the bioassay technique used by Tenney to determine catecholamine concentration is quite different from the direct chemical quantitation of ACO performed in our study. Tenney used the response of the nictitating membrane as an index of circulating catecholamines and it is possible that this response might be altered in the spinal animal, which may have become severely acidotic and deteriorated.

Our findings are consistent with, and may possibly explain, the observations of Manger *et al*(6) and more recently of Darby and Watts(2), who reported that during acute hemorrhagic hypotension in intact dogs there

TABLE III. Effect of Acute Hypoxia on ACO (Mean Values 6 Animals).

		pH	BE*	PaO_2 (mm Hg)	$PaCO_2$ (mm Hg)	BP (mm Hg)	BG†	ACO‡
#1	Normoxia	7.46	−4.6	100	26	94	97	.6
#2	Hypoxia	7.49	−7.1	36	21	86	89	.7
#3	Normoxia	7.42	−6.5	105	26	105	90	.7

* BE, base excess in mEq/l. † BG, blood glucose in mg/100 ml. ‡ ACO, mean adrenal catecholamine output in mμg/kg/min.

Analysis of variance performed on the logarithms of the individual ACO values indicates no significant difference between Nos. 1, 2, and 3.

was a marked initial rise in peripheral arterial catecholamine levels followed by a secondary increase as hypotension was maintained and acidosis developed. Darby and Watts also found that the initial rise in catecholamine concentration was blocked by extradural procaine anesthesia of the spinal cord, but an increase occurred subsequently with the onset of acidosis. Thus, stimuli, other than those transmitted from baro-receptors of the carotid sinus, aortic arch and the vasomotor centers, may cause liberation of the catecholamines from the adrenal medulla.

Our results suggest, as has been proposed by Morris and Millar(8), that in the dog, hypercapnic acidosis might stimulate directly adrenal medullary secretion by a non-neural mechanism. These investigators measured norepinephrine and epinephrine concentrations in peripheral arterial blood samples in anesthetized intubated dogs breathing 100% O_2, or 10% and 30% CO_2 in O_2. In one group in addition to an infusion of hexamethonium, complete adrenal denervation was undertaken by splanchnisectomy plus excision of the abdominal sympathetic chains. These dogs, while showing no increase in plasma catecholamines with a pH fall from 7.5 to 7.02, did show a significant increase in catecholamine concentration (mainly epinephrine) when the pH was further reduced to 6.71. The magnitude of this response was approximately one-third that observed in non-operated dogs under similar conditions. They concluded that at pH levels below 6.8 an increased CO_2 or hydrogen ion concentration directly stimulates catecholamine release from the adrenal medulla and possibly, in addition, from extra-adrenal sympathetic receptor sites. Our experiments, while in general agreement with the former conclusion of these investigators, suggest that CO_2 directly stimulates medullary secretion above pH 6.8. Although the two highest individual adrenal catecholamine outputs occurred with a pH below 6.8, nevertheless ACO was significantly increased in the pH range of 6.85 to 6.95. This difference might be explained by the fact that in this study ACO was determined which might be a more selective and sensitive measurement of adrenal medullary secretion than peripheral arterial catecholamine concentrations.

A moderate fall in blood pressure was observed during hypercapnia and might be attributed to the acidosis which both decreases peripheral vascular resistance(2) and reduces the strength of cardiac contraction(10). However, when the adrenal gland was separated from the centrally located sympathetic centers, hypotension could not stimulate the adrenals through a reflex originating in the baro-pressor areas of the carotid sinus and aortic arch. Furthermore, it has been shown previously that acute hypotension following hemorrhage and unaccompanied by acidosis will not increase adrenal catecholamine output in the animal with C_8 spinal cord section (11).

When hypercapnic acidosis was corrected by administration of THAM and pH restored to normal levels, adrenal catecholamine output decreased but remained above control values. The previously described(14) hypoglycemic effect of THAM was also seen.

The close similarity of the changes in adrenal venous and peripheral arterial acid-base measurements confirms earlier observations by Mittelman and co-workers(7) that the pO_2 and pCO_2 of adrenal venous blood (rather than peripheral venous blood) approximates that of peripheral arterial blood. The hyperglycemic effect of epinephrine was observed since the blood glucose levels paralleled changes in ACO.

The fact that THAM decreased ACO in the presence of continuous CO_2 retention might indicate that it is an increase in hydrogen ion concentration rather than CO_2 accumulation *per se* which plays a major role in stimulating adrenal medullary secretion. Indeed, *in vitro* studies(3) using perfused ox spleen have shown that addition of a variety of acids to the perfusate produced a marked increase in norepinephrine release with a drop in perfusate pH from 6.9 to 6.6. The amount of norepinephrine released was related to the decrease in pH and not the type of acid employed to achieve that level. Similarly, von Euler and Lishajka(18) have shown *in vitro* an inverse relationship between pH (range 7.0 to 8.5) and depletion

rate of norepinephrine from storage granules of bovine adrenergic splenic nerves.

The precise location where the increased hydrogen ion concentration might exert its effect was not discussed. Barring the release by acidosis of a humoral factor capable of stimulating the medullary cells of the adrenals, it seems that an increase in hydrogen ion concentration might stimulate catecholamine release from the adrenal gland as it does from the sympathetic nerves of the isolated spleen(3). The mechanism involved in this release remains to be determined. In the present experiments the adrenal glands were not isolated from their spinal and splanchnic connections. It is not possible, therefore, to attribute the release of catecholamine from the adrenal gland to a direct effect of $[H^+]$. The stimulating effect of increased $[H^+]$ on catecholamine release contrasts with the lack of response caused by hypoxia.

Conclusion. In 8 spinal dogs adrenal blood flow and adrenal catecholamine output (ACO) were measured. Hypoxia (PaO_2 36 mm Hg) without acidosis did not change significantly ACO, while hypercapnic acidosis ($PaCO_2$ 127, pH 6.93) produced a significant increase in ACO. These findings confirm previous observations indicating that acidosis more than hypoxia stimulates catecholamine release.

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Erythropoietin Effects on Fasted Rats as a Function of Time of Injection.* (31155)

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It has been suggested that erythropoietin (EP)-containing extracts not only produce differentiation of stem cells into erythrocyte precursors(1) but also affect the precursors already present at time of injection(2,3,4,5), stimulating Hb synthesis and the release of reticulocytes(6). The purpose of the experiments described here was to evaluate the ef-

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