NOREPINEPHRINE RELEASE DURING RESPIRATORY ACIDOSIS IN ADRENALECTOMIZED DOGS

R. A. Millar, M.D., M.Sc., and M. E. Morris, M.D., C.M.

Sympatho-adrenal stimulation accompanying severe respiratory acidosis was recently demonstrated by the measurement of progressive increases in plasma epinephrine and norepinephrine in dogs subjected to the condition of apneic oxygenation ("diffusion respiration").[1] An early rise, predominantly in plasma norepinephrine, was followed by pronounced increases in the levels of both catecholamines, epinephrine being greatly increased at extreme levels of arterial P_{CO_2}. The possibility that the initial rise in plasma norepinephrine was due not to adrenal medullary secretion but to liberation of this amine from extraadrenal sites or organs[2, 3] has been investigated in the present study, in which plasma norepinephrine (and epinephrine) levels have been determined in adrenalectomized dogs subjected to steady and moderately severe degrees of respiratory acidosis.

Method

Six experiments were performed. Dogs were anesthetized with intravenous thiopental, the trachea was intubated with a no. 10 cuffed Magill tube, and intermittent positive pressure ventilation (+10 to 15 cm. H_2O) was begun with oxygen, using the Bird respirator (Marks 4 and 8) and a Ruben nonrebreathing valve. An intravenous infusion of succinylcholine chloride (0.1 per cent) was given at a slow rate for the duration of each experiment, and light anesthesia was maintained with small increments of thiopental.

The right and left adrenal glands were then removed through bilateral loin incisions, care being taken to ensure complete excision with a minimum of blood loss. Pulmonary ventilation with oxygen was continued for 45–60 minutes after adrenalectomy, and in this period a femoral artery was cannulated for removal of blood samples and recording of blood pressure, using a Statham gauge, Model P23A, and Sanborn recorder. In 4 experiments dextran 6 per cent in saline was given intravenously to replace blood loss.

Control blood samples were then withdrawn and ventilation of the lungs with 20 per cent carbon dioxide in oxygen was begun. Further blood samples were withdrawn at intervals of 15, 30, 60, and 120 minutes, during which time a moderately steady state of respiratory acidosis was maintained. The increased arterial P_{CO_2} was then lowered by ventilation of the lungs with oxygen for 15 minutes, after which a final blood sample was withdrawn. An equal volume of normal saline was replaced after each blood sampling.

Blood for assay of norepinephrine and epinephrine was withdrawn into tubes containing a few drops of heparin (1,000 units/ml.). After centrifuging, the plasma was aspirated and applied to alumina columns, the extraction procedure then being completed within a few hours. Epinephrine and norepinephrine in the plasma eluates were determined by the trihydroxyindole method[4] essentially as described previously,[5] but with the addition of 0.5 ml. of 1 per cent disodium ethylenediaminetetra-acetate to the final mixture before measurement of fluorescence. Errors within ± 25 per cent are involved in the differential estimation of epinephrine and norepinephrine in single plasma samples, and the values, which refer to μg. free base/l. of plasma, are uncorrected for losses in recovery up to 30 per cent.

Blood for *p*H, P_{CO_2}, and "standard" bicarbonate estimations (8 ml.) was withdrawn anaerobically into syringes moistened with heparin. Whole blood *p*H, and *p*H of separated plasma equilibrated at 38 C. with known concentrations of carbon dioxide, were determined by means of the Radiometer *p*H meter and Astrup apparatus.[6] From these *p*H determinations the "standard" bicarbonate in

Accepted for publication September 15, 1960.
The authors are in the Department of Anaesthesia, Montreal Neurological Institute and McGill University, Montreal, P. Q., Canada.

TABLE 1

PLASMA NOREPINEPHRINE AND EPINEPHRINE, WHOLE BLOOD pH, AND ARTERIAL P_{CO_2} AND "STANDARD" BICARBONATE IN ADRENALECTOMIZED DOGS DURING 120 MINUTES OF VENTILATION * WITH 20 PER CENT CO_2 IN O_2 (6 EXPERIMENTS)

	Time (minutes)	Epinephrine (μg./l.)	Norepinephrine (μg./l.)	pH	P_{CO_2} (mm. Hg)	Standard HCO_3^- (m.M/l.)
Control	—	0	0.06	7.40	34	20
20% CO_2 in O_2	+15	0.62	1.1	6.95	133	24
	+30	0.27	0.89	6.95	135	24
	+60	0.47	0.99	6.94	138	24
	+120	0.22	0.95	6.85	176	25
100% O_2	+15	0	0.40	7.29	43	19
Control	—	0	0	7.60	17	18
20% CO_2 in O_2	+15	0	0	6.90	158	25
	+30	0	0.63	6.89	156	24
	+60	0.04	0.57	6.87	162	24
	+120	0	0.69	6.85	175	24
100% O_2	+15	0	0	7.35	38	20
Control	—	0.10	0.37	7.56	26	23
20% CO_2 in O_2	+15	0.04	0.62	6.99	140	28
	+30	0.25	1.2	6.98	144	28
	+60	0.46	1.4	6.97	128	24
	+120	0.07	1.3	6.95	154	28
100% O_2	+15	0.05	0.81	7.35	48	24
Control	—	0	0.37	7.48	30	21
20% CO_2 in O_2	+15	0.31	1.0	6.92	164	27
	+30	0.07	1.1	6.92	158	26
	+60	0.14	1.9	6.92	148	25
	+120	0.80	2.1	6.89	175	27
100% O_2	+15	0	0.92	7.37	40	22
Control	—	0.02	0.09	7.46	41	27
20% CO_2 in O_2	+15	0.13	0.14	6.99	150	30
	+30	0.21	1.9	6.99	148	30
	+60	0.42	1.3	6.99	165	34
	+120	0.31	1.0	6.99	164	33
100% O_2	+15	0.22	0.81	7.43	44	27
Control	—	0	0.23	7.52	24	20
20% CO_2 in O_2	+15	0	1.2	7.00	135	28
	+30	0.15	0.75	6.98	137	27
	+60	0.17	0.62	6.97	148	28
	+120	0.32	1.1	6.92	152	25
100% O_2	+15	0	0.24	7.37	36	20

* The period of respiratory acidosis was preceded and followed by ventilation with oxygen.

separated plasma, and the CO_2 tension, were calculated as described by Astrup.[7]

RESULTS

Table 1 presents the plasma norepinephrine and epinephrine levels, with values for whole blood *p*H, arterial P_{CO_2}, and "standard" bicarbonate before, during, and after the 120-minute period of respiratory acidosis, in all six experiments. The average levels of plasma norepinephrine, arterial P_{CO_2}, and mean arterial blood pressure (diastolic + ⅓ pulse pressure) are shown in figure 1.

In the control samples withdrawn 45–60 minutes after bilateral adrenalectomy, plasma norepinephrine averaged 0.19 μg./l. and a moderate respiratory alkalosis was usually present because of pulmonary overventilation

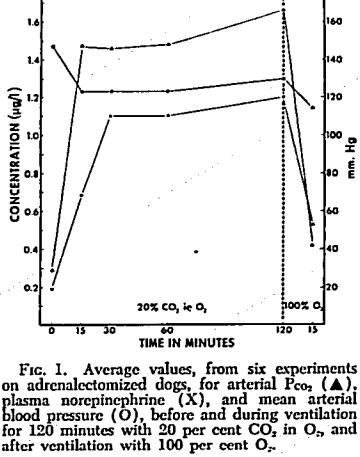

FIG. 1. Average values, from six experiments on adrenalectomized dogs, for arterial P_{CO_2} (▲), plasma norepinephrine (X), and mean arterial blood pressure (O), before and during ventilation for 120 minutes with 20 per cent CO_2 in O_2, and after ventilation with 100 per cent O_2.

(table 2). The acute respiratory acidosis then produced by ventilation with 20 per cent carbon dioxide in oxygen is shown by the subsequent fall in *p*H, rise in P_{CO_2}, and moderate increase in "standard" bicarbonate, the levels reached after 15 minutes remaining relatively constant throughout the two hour period studied (table 2 and fig. 1).

After 15 minutes, when average P_{CO_2} had increased from 29 to 147 mm. of mercury, plasma norepinephrine showed definite rises in 4 studies. The average level in the six experiments, 0.68 μg./l., was significantly higher than the control ($P < 0.05$). In the 30-minute sample, at a P_{CO_2} of 146 mm. of mercury, plasma norepinephrine was 1.1 μg./l. After 60 and 120 minutes of respiratory acidosis, P_{CO_2} levels of 148 and 166 mm. of mercury, plasma norepinephrine averaged 1.1 and 1.2 μg./l. respectively (table 2 and fig. 1). These increases were all highly significant ($P < 0.01$).

Variable rises in plasma epinephrine, usually too small for accurate assessment, were measured during the period of respiratory acidosis from an average control level of 0.02 μg./l. to 0.18, 0.16, 0.28, and 0.29 μg./l. after 15, 30, 60 and 120 minutes respectively. Because of small errors in the differential estimation of norepinephrine and epinephrine, these changes in plasma epinephrine, although significant statistically in the 30, 60 and 120 minute samples, cannot definitely be considered meaningful. It is a distinct possibility, however, that small increases in plasma epinephrine may occur subsequent to bilateral adrenalectomy because of the presence of this amine in various extraadrenal areas.[8]

Mean arterial blood pressure was reduced during respiratory acidosis, largely due to a consistent fall in diastolic pressure (table 2 and fig. 1); the effects on systolic pressure were variable. Heart rate was reduced. Lowering

TABLE 2

AVERAGE PLASMA NOREPINEPHRINE AND EPINEPHRINE LEVELS WITH ARTERIAL BLOOD PRESSURE AND HEART RATE (6 EXPERIMENTS ON ADRENALECTOMIZED DOGS) DURING 120 MINUTES OF VENTILATION WITH 20 PER CENT CO_2 IN O_2*

	Time (minutes)	pH	P_{CO_2} (mm. Hg)	Standard HCO_3^- (mM/l.)	Epinephrine (μg./l.)	Norepinephrine (μg./l.)	Arterial Blood Pressure (mm. Hg) Syst.	Arterial Blood Pressure (mm. Hg) Diast.	Heart Rate
Control	—	7.50	29	22	0.02	0.19	187	127	180
20% CO_2 in O_2	+15	6.96	147	27	0.18	0.68	176	96	163
	+30	6.95	146	27	0.16	1.1	173	98	151
	+60	6.94	148	26	0.28	1.1	171	99	137
	+120	6.91	166	27	0.29	1.2	179	106	131
100% O_2	+15	7.36	42	22	0.05	0.53	143	98	167

* The period of respiratory acidosis was preceded and followed by ventilation with oxygen.

of arterial P_{CO_2} to normal resulted in a fall in mean pressure, because of a fall in systolic pressure; changes in diastolic pressure were inconsistent. Severe circulatory impairment, or persistent cardiac arrhythmias, did not occur in any experiment during or following the two-hour period of respiratory acidosis.

Ventilation with 100 per cent oxygen reduced plasma norepinephrine concentration in every experiment, although basal levels were not regained. The fall in plasma norepinephrine, from 1.2 μg./l. at a P_{CO_2} of 166 mm. of mercury, to 0.53 μg./l. at a P_{CO_2} of 42 mm. of mercury was significant ($P < 0.05$). The final average value of 0.53 μg./l. (measured at the conclusion of the experiments) did not differ significantly from the initial control level of 0.19 μg./l. (measured before respiratory acidosis).

Discussion

The results of these experiments, which show that significant increases in plasma norepinephrine occur in adrenalectomized dogs when the arterial P_{CO_2} is increased, indicate that norepinephrine is liberated from sympathetic nerves (or other extraadrenal sites which contain norepinephrine) during an acute respiratory acidosis. Interference with the metabolic transformation of catecholamines may also be related to increases in plasma norepinephrine at reduced levels of blood and tissue *p*H.

These experiments follow the previous measurement of a predominant early increase in plasma norepinephrine as the arterial carbon dioxide tension rises during the initial phase of apneic oxygenation[1] when arterial oxygen saturation is well maintained. Other studies have also shown that pronounced increases in plasma norepinephrine during apneic oxygenation are not prevented by bilateral adrenalectomy, a procedure which almost completely abolishes the rise in plasma epinephrine (Millar and Morris—unpublished data). It seems evident, therefore, that substantial amounts of norepinephrine can be released from areas outside the adrenal medulla during respiratory acidosis.

Previous studies in adrenalectomized dogs demonstrated gradual but small increases in plasma norepinephrine during graded hemorrhagic hypotension.[5] In these earlier experiments the final norepinephrine level, measured just prior to circulatory collapse, was 0.58 μg./l., in contrast to an average maximum of 1.4 μg./l. during moderately severe respiratory acidosis in the present study. In intact dogs, hemorrhage did not result in marked increases in plasma norepinephrine until the later stages,[9] whereas during carbon dioxide accumulation the concentration of norepinephrine was initially higher than that of epinephrine.[1]

Thus, while sympatho-adrenal stimulation is a feature both of respiratory acidosis and of hemorrhagic hypotension, the mechanisms and effects of these two stimuli are not the same. Respiratory acidosis apparently results in the release of norepinephrine from extraadrenal areas at an early stage, this being reinforced by adrenal medullary secretion which later becomes more pronounced as the degree of acidosis increases.[1] Hemorrhagic hypotension differs by predominantly inducing epinephrine release from the adrenal medulla, norepinephrine being liberated in smaller amounts from the adrenal gland, and from extraadrenal areas in the later stages.

Release of norepinephrine from sympathetic nerve endings, or from organs containing this amine, may have an important bearing on the occurrence of circulatory disturbances during and following the increases in arterial P_{CO_2} which can accompany general anesthesia. It seems likely, for example, as suggested by infusion experiments,[10] that cardiac arrhythmias are more dependent on the local release of sympathetic transmitter substance at nerve endings within the heart, than to an increase in circulating catecholamine levels, whether resulting from exogenous administration or by endogenous liberation from the adrenal medulla.

Summary

Respiratory acidosis was induced in adrenalectomized dogs lightly anesthetized with pentothal and paralyzed with succinylcholine, by intermittent positive pressure ventilation with 20 per cent carbon dioxide in oxygen. Consistent rises in plasma norepinephrine concentration were measured at increased levels of arterial P_{CO_2}. Plasma epinephrine showed variable increases, too small for accurate assess-

ment. It is concluded that substantial amounts of norepinephrine can be released from areas outside the adrenal medulla during an acute respiratory acidosis.

These studies were partly supported by a grant from Parke, Davis & Co., and the Cooper Fund, McGill University Faculty of Medicine.

REFERENCES

1. Millar, R. A.: Plasma adrenaline and noradrenaline during diffusion respiration, J. Physiol. 150: 79, 1960.
2. Vogt, M.: Concentration of sympathin in different parts of central nervous system under normal conditions and after administration of drugs, J. Physiol. 123: 451, 1954.
3. Outschoorn, A. S., and Vogt, M.: Nature of cardiac sympathin in dog, Brit. J. Pharmacol. 7: 319, 1952.
4. Euler, U. S. v. and Floding, I.: Fluorimetric micromethod for differential estimation of adrenaline and noradrenaline, Acta physiol. scand. (supp. 118) 33: 46, 1955.
5. Millar, R. A., Keener, E., and Benfey, B. G.: Plasma adrenaline and noradrenaline after phenoxybenzamine administration and during haemorrhagic hypotension, in normal and adrenalectomized dogs, Brit. J. Pharmacol. 14: 9, 1959.
6. Astrup, P. and Schrøder, S.: Apparatus for anaerobic determination of the pH of blood at 38 degrees centigrade, Scand. J. Clin. Lab. Invest. 8: 30, 1956.
7. Astrup, P.: Simple electrometric technique for determination of carbon dioxide tension in blood and plasma, total content of carbon dioxide in plasma, and bicarbonate content in "separated" plasma at a fixed carbon dioxide tension (40 mm. Hg.), Scand. J. Clin. Lab. Invest. 8: 33, 1956.
8. Euler, U. S. v.: Nature of adrenergic nerve mediators, Pharmacol. Rev. 3: 247, 1951.
9. Millar, R. A., and Benfey, B. G.: Fluorimetric estimation of adrenaline and noradrenaline during haemorrhagic hypotension, Brit. J. Anaesth. 30: 159, 1958.
10. Price, H. L., Lurie, A. A., Jones, R. E., Price, M. L., and Linde, H. W.: Cyclopropane anesthesia; epinephrine and norepinephrine in initiation of ventricular arrhythmias by carbon dioxide inhalation, ANESTHESIOLOGY 19: 619, 1958.

INTRATHECAL PHENOL Intrathecal phenol-glycerin mixture was used in 31 patients for the relief of painful spasticity of the lower limbs. Twenty-one of these patients had multiple sclerosis. Small amounts in small increments were used with patients carefully positioned so that the second to fourth lumbar interspace was lowest. Paresthesias on the side selected were sought, the needle deliberately being placed laterally. Correctness in placement of the needle was indicated by patients reporting warmness and tingling in the thigh and knee after the first increment was injected. Paresthesias of the ankle or buttock indicated incorrect placement and were indications for discontinuing the procedure and trying again another day. Urinary retention occurred and did not clear in 2 patients. No patients were made completely immobile who had been previously mobile. Relief of painful spasm usually lasted 6–12 months. (*Leversedge, L. A. and Maher, R. M.: Use of Phenol in Relief of Spasticity, Brit. Med. J. 2: 21 (July 2) 1960.*)

INTRATHECAL PHENOL Phenol and glycerin (1 : 20) has been injected for the relief of pain in malignancy and in the treatment of pain of nonmalignant origin such as might be caused by flexor spasms, parkinsonian rigidity, arthritis, spondylitis, and failed disc operations. Such therapy is effective for lumbosacral and dorsal sites below the third thoracic vertebra (D3). Relief was complete in 61 of 81 cases. More recently, using a gradation method of injection control, relief has been attained in 12 of 14 cases. Above D3 intrathecal phenol is not used. (*Maher, R. M.: Further Experiences with Intrathecal and Subdural Phenol, Lancet 1: 895 (April 23) 1960.*)