Comparative Effects of Hypoxia, Adrenocorticotropin and Methylcholine on Adrenocortical Secretory Rates[1] (36902)

S. F. MAROTTA

Department of Physiology, University of Illinois College of Medicine, School of Basic Medical Sciences, Chicago, Illinois 60680

Activation of the hypothalamo–hypophyseal–adrenocortical (HHA) system occurs when animals are acutely exposed to hypoxic gas mixtures (1–3); however, the rapidity with which this stressor is capable of stimulating the HHA system has not been thoroughly investigated. Hypoxemia, which does not directly stimulate any portion of this system, requires the activation of peripheral respiratory chemoreceptors (4, 5), which in turn by some undetermined neural pathway (6) probably stimulate the secretion of the corticotropin-releasing factor (CRF) from the hypothalamic median eminence. Recently it has been shown that the adrenocortical secretory rates of intact anesthetized dogs begin to rise within 1 to 2 min after breathing 10% oxygen (O_2) at ground level, and attain maximal levels within 5 to 10 min. On the other hand, some investigators believe that the adrenal cortex requires several min of exposure to adrenocorticotropin (ACTH) before noticeable elevations in secretory rates occur (7–9), and the latter are affected by adrenal blood flow (7–10). Since hypoxemia is dependent upon the proper functioning of many anatomical structures for its effect on the adrenal cortex, it seemed instructive to compare the effects of acute hypoxia with the infusions of ACTH and acetyl-β-methylcholine (MEC), a substance which supposedly stimulates the central pole of the hypothalamus (11, 12), on the rapidity and degree with which these agents affect adrenocortical secretory rates and lumboadrenal vein blood flow.

Materials and Methods. Fifteen male mongrel dogs, weighing between 12 and 15 kg, were equally divided into 3 groups. The animals were anesthetized with sodium pentobarbital (30 mg/kg) and prepared as previously described for the collection of left lumboadrenal vein blood and anaerobic femoral arterial blood (13). In addition, a polyethylene cannula was guided through the right external jugular vein into the right atrium for the infusion of fluids or drugs. Respiratory and pulse rates were monitored continuously on a Grass polygraph (Model 5). Supplemental doses of the anesthetic were administered as required.

Four hr after the completion of surgery, 2000 IU of heparin were infused through the jugular cannula. The lumboadrenal vein cannula was opened while blood, which normally flows into the inferior vena cava, was shunted into the cannulated vessel by occluding the adrenal vein. The adrenal venous blood was collected continuously in graduated conical test tubes at 1 min intervals for 5 min immediately prior to the induction of hypoxia by breathing 10% O_2 in nitrogen or the rapid (5–10 sec) infusions of 1.5 U ACTH (Armour Pharmaceutical Co.)/kg body weight or 20 μg MEC (Merck & Co., Inc.)/kg into the right atrium. Adrenal venous blood was collected during the initiation of hypoxia and the rapid infusion of drugs, and this sample was considered the first min sample. Additional samples were collected continuously at 1 min intervals from 2 to 7 min as well as at 10 and 15 min. Anaerobic arterial blood, which was withdrawn midway during the 0, 1, 5 and 15 min sampling periods, was immediately analyzed for Pa_{O_2}, Pa_{CO_2} and pH with a blood gas analyzer (Instrumentation Laboratory, Inc.). To avoid reductions in vascular volumes

[1] Supported in part by the Office of Naval Research Contract NR 101-580.

TABLE I. Effects of Breathing 10% O_2 (HYP), Adrenocorticotropin (ACTH) and Acetyl-β-methylcholine Chloride (MEC) on Various Cardiopulmonary Measurements.

	HYP (min)			ACTH (min)			MEC (min)		
Measurement[a]	1	5	15	1	5	15	1	5	15
Resp. rate (min)	7 (1)[b]	21 (4)	28 (6)	1 (1)	3 (2)	4 (2)	−3 (3)	15 (6)	16 (7)
Pulse rate (min)	4 (1)	13 (2)	15 (2)	1 (2)	−2 (5)	1 (5)	−69 (20)	−3 (9)	−1 (7)
Pa_{O_2} (mm Hg)	−15.0 (1.5)	−39.1 (5.8)	−42.0 (5.8)	0.6 (1.1)	−3.2 (2.1)	2.8 (1.3)	−5.2 (1.0)	−19.0 (4.9)	−3.4 (3.0)
Pa_{CO_2} (mm Hg)	−4.7 (0.9)	−11.6 (2.1)	−15.8 (1.8)	0.4 (0.5)	0.1 (0.8)	−0.9 (1.0)	1.0 (0.7)	3.4 (2.3)	−1.0 (2.0)
Art. pH	0.018 (0.006)	0.073 (0.006)	0.085 (0.008)	−0.001 (0.001)	0.001 (0.011)	−0.002 (0.007)	−0.003 (0.002)	−0.034 (0.011)	−0.002 (0.009)

[a] Values reported as mean difference from control (zero time).

[b] Standard error in parentheses.

during the collection period (20 min), the animals were infused with 6% dextran in saline at rates which approximated very closely the amount of blood withdrawn. Upon completion of the experiment the cannulated adrenal gland was excised, cleaned and accurately weighed.

The volumes of lumboadrenal vein blood per minute were recorded prior to performing microhematocrits and centrifuging the remainder of the blood at moderate speeds. The plasma was removed and stored in the freezer (— 25°) until analyzed for 11-hydroxycorticosteroids (11-OHCS) by the fluorometric method of Kitabchi and Kitchell (14).

Results. The arterial pH and blood gases of all animals prior to the induction of hypoxia and the infusion of drugs varied from 7.390 to 7.429 for pH, 78.9 to 85.1 mm Hg for Pa_{O_2}, and 37.9 to 43.4 mm Hg for Pa_{CO_2}. Breathing 10% O_2 caused rapid (within 1 min) and significant ($p < .05$) increases in both respiratory and pulse rates at 1, 5 and 15 min (Table I). Concomitant with these changes, Pa_{O_2} and Pa_{CO_2} decreased significantly ($p < .05$) throughout the experimental period and reached values of 42.0 ± 5.8 and 15.8 ± 1.8 mm Hg, respectively, below control levels at 15 min. The marked increases in arterial pH paralleled the changes in Pa_{CO_2} and the elevations in respiratory rates. Although the infusion of ACTH caused no noticeable effects on the various cardiopulmonary measurements, MEC exhibited pronounced effects (Table I). During the first minute it caused temporary decreases in respiratory and pulse rates, as well as in arterial O_2 tension and pH. The pulse rates returned to preinfusion levels within 5 min, but the animals showed marked ($p < .05$) hypoxemia (—19.0 mm Hg Pa_{O_2}) and a mild acidosis (pH = —0.034) in the presence of hyperventilation (+15 breaths/min). All of these parameters, however, returned towards control levels by the time the last adrenal blood sample was collected.

The 11-OHCS secretory rates of all dogs with mean adrenal gland weights of 0.81 ± 0.11 g ranged from 3 to 7 μg/min/g before the beginning of the experimental period. Upon the initiation of hypoxia secretory rates increased ($p < .05$) within the first minute (Fig. 1). These increases were due not only to slight elevations in adrenal blood flow but more so to increases in 11-OHCS concentrations (μg/ml) in the plasma of 4 of the 5 dogs. Calculations (Table II), based upon fitting (least squares) the data collected during the first 5 min and corrected for zero time levels to the equation $Y = a + bX$, showed that 11-OHCS secretory rates increased 0.66 μg/min/g while plasma flow increased only 0.12 ml/min/g. Whereas

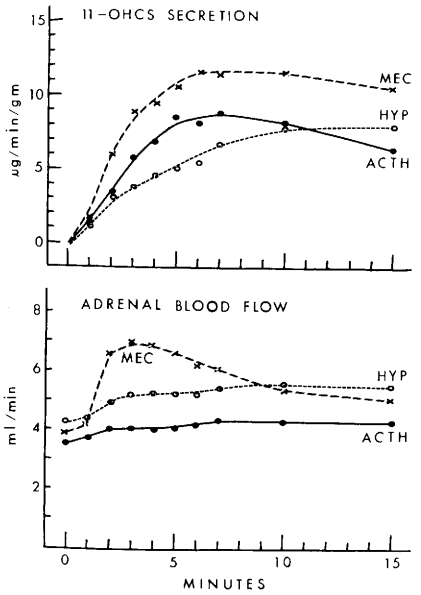

FIG. 1. The effects of breathing 10% O_2 (HYP), or the rapid infusion of adrenocorticotropin (ACTH) or acetyl-β-methylcholine (MEC) on the adrenocortical 11-OHCS secretory rates (corrected for control rates) and lumboadrenal vein blood flow of anesthetized dogs.

adrenal blood and plasma flows remained relatively constant after 5 min of hypoxia, the 11-OHCS rates continued to rise reaching maximal rates at 7 to 10 min and were maintained for the duration of the experiment (Fig. 1).

Immediate (1 min) increases in 11-OHCS rates were observed after the rapid infusion of ACTH in all dogs. At this time 4 of the 5 dogs exhibited marked increases in 11-OHCS concentrations while showing only minor elevations in adrenal blood and plasma flows. The 11-OHCS rates, which rose 1.70 μg/min/g for the first 5 min (Table II), were much faster than those observed in hypoxic dogs. Maximal secretory rates resulting from ACTH infusion were observed between 5 and 7 min before gradually declining (Fig. 1).

Of the 5 dogs in the MEC group only 3 exhibited marked increases in both 11-OHCS concentrations and rates during the first min, while the remaining 2 dogs showed small drops in blood flow. However, 11-OHCS rates increased very rapidly (2.14 μg/min/g) during the first 5 min reaching maximal rates at 5–7 min after the infusion of MEC when blood flow had already begun to decline (Fig. 1). The 0.34 ml/min/g increase in plasma flow was the largest of the 3 groups (Table II). In addition, this was the only group of animals in which a significant ($p < .05$) coefficient of correlation (.5994) between 11-OHCS (μg/min/g) and plasma flow (ml/min/g) was observed.

Discussion. The results of these experiments show that the 11-OHCS secretory rates of intact anesthetized dogs made hypoxic or intravenously infused with ACTH or MEC begin to increase in most dogs within a min and are clearly elevated in all dogs by 2 min. Longer delay times of at least 2 min in the isolated perfused dog's adrenal gland (7–9), or of 5 to 15 min in the *in situ* adrenal preparation (8) of rats (hypophysectomized 2 hr previously) have been reported for ACTH (constant adrenal blood flow) to affect adrenocortical secretions. In the present study significant increases in 11-OHCS concentrations and rates were noted in the first min samples collected after the rapid intraatrial infusion of ACTH. Some of this discrepancy in delay times could conceivably be accounted for by the experimental models employed. It may be that the time as well as the manipulations involved in isolating and connecting the adrenal gland to a perfusion system affect the response of the adrenal cortex to ACTH. It has been shown that adrenal venous congestion or the temporary interruption of the blood supply to the gland not only lowers the secretory rates (15), but also depresses markedly the adrenal response to ACTH (16). In addition, it is

TABLE II. Rates at Which 11-OHCS and Plasma Flow Increased During the First 5 Min of Hypoxia (HYP), or the Infusion of Adrenocorticotropin (ACTH) or Acetyl-β-methylcholine (MEC).

Group	11-OHCS (μg/min/g)[a]	Plasma flow (ml/min/g)[a]
HYP	0.66 ± 0.16[b]	0.12 ± 0.04[b]
ACTH	1.70 ± 0.46[b]	0.10 ± 0.09
MEC	2.14 ± 0.78[b]	0.34 ± 0.15[b]

[a] Mean ± SE.
[b] $p < .05$.

possible that depriving the adrenal glands of sufficient ACTH even for a short time may lengthen the delay time for steroid biosynthetic mechanisms to again respond to ACTH. Needless to say, the animals used herein did not have low or depleted circulating titers of ACTH since control 11-OHCS rates ranged from 3 to 7 μg/min/g, whereas basal rates of less than 2 μg/min have been found in undisturbed conscious dogs in which the lumboadrenal vein was cannulated on the side which had been previously desensitized by severing the dorsal roots from T_{10}–L_3 (2).

There is little doubt that adrenocortical secretory rates are dependent to some extent upon adrenal blood flow (7–10). The results of hypoxia and MEC, which are known to affect the cardiovascular system, substantiate these findings. The sudden increase in adrenal blood flow may rapidly release preformed steroids stored within the adrenal gland. Increases in flow alone, however, could not account for these elevated 11-OHCS rates, since in the hypoxic animals the increase in flow was small when compared to the marked elevations in secretory rates, while in the MEC group flow had already begun to decrease before peak secretory rates had been reached. Furthermore, L'Age, Gonzalez-Luque and Yates (10) have shown that in dexamethasone treated dogs MEC caused an increased adrenal blood flow without appreciably affecting cortisol secretion. The latter indicates that this drug does not act directly on the adrenal cortex but in all probability affects the hypothalamo–hypophyseal complex to release ACTH (11, 12).

The stressor hypoxia requires special consideration since as noted previously, functioning peripheral chemoreceptors are necessary to activate the HHA system. Since Fitzgerald, Leitner and Liaubet (17) have shown that hypoxic blood caused maximal afferent impulse frequency from the carotid nerve within 3.4 sec (range 1–6 sec), it is not unreasonable to assume that within less than a min hypoxia can stimulate the hypothalamo–hypophyseal complex to release ACTH, which in turn, is transported via the systemic circulation to the adrenal cortex. While these events are occurring hypoxia, which markedly excites the cardiopulmonary system, not only increases adrenal blood flow but also decreases the transit time of ACTH from the adenohypophysis to the adrenal cortex resulting in rapid and sustained increases in adrenocortical secretory rates.

Summary. Lumboadrenal vein blood was continuously collected at min intervals from anesthetized dogs who were either made hypoxic or infused with ACTH or MEC. The secretions of 11-OHCS increased within the first min and attained peak rates between 5 and 10 min of exposure to these agents. Part of the increased secretory rates of the hypoxic and MEC groups could be accounted for by increases in adrenal blood flow. These data suggest that a min is sufficient time for hypoxia to (a) activate the hypothalamo–hypophyseal complex to release ACTH, (b) decrease the transit time for ACTH to reach the adrenal gland, and (c) increase adrenal blood and plasma flows. When occurring simultaneously, these 3 factors result in a rapid and sustained increase in adrenocortical secretory rates during hypoxia.

The excellent technical assistance of E. Blomquist, C. Lau and L. Malasanos is greatly appreciated. Special thanks are due Dr. G. Courtney for encouragement, assistance and discussions during the course of this investigation.

1. Hirai, K., Atkins, G., and Marotta, S. F., Aerosp. Med. **34**, 814 (1963).

2. Marotta, S. F., Hirai, K., and Atkins, G., Proc. Soc. Exp. Biol. Med. **114**, 403 (1963).

3. Marotta, S. F., Hirai, K., and Atkins, G., Proc. Soc. Exp. Biol. Med. **118**, 922 (1965).

4. Lau, C., and Marotta, S. F., Aerosp. Med. **40**, 1065 (1969).

5. Lau, C., and Marotta, S. F., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **29**, 778 (1970).

6. Lau, C., Amer. J. Physiol. **221**, 607 (1971).

7. Urquhart, J., Amer. J. Physiol. **209**, 1162 (1965).

8. Porter, J. C., and Klaiber, M. S., Amer. J. Physiol. **209**, 811 (1965).

9. Yates, F. E., and Brennan, R. D., *in* "Hormonal Control Systems" (E. B. Stear and A. H. Kadish, eds.), Suppl. 1, p. 20. Amer. Elsevier, New York (1969).

10. L'Age, M., Gonzalez-Luque, A., and Yates, F. E., Amer. J. Physiol. **219**, 281 (1970).

11. Soulairac, A., Schaub, C., Franchemont, P.,

Aymaid, N., and Van Couwenberge, H., Ann. Endocrinol. **29**, 45 (1968).

12. Soulairac, A., and Soulairac, M.-L., Acta Neurol. Belg. **69**, 539 (1969).

13. Marotta, S. F., Proc. Soc. Exp. Biol. Med. **141**, 915 (1972).

14. Kitabchi, A. E., and Kitchell, L. C., Anal. Biochem. **24**, 529 (1970).

15. Monos, E., Koltay, E., Sulyok, A., Venkey, T., and Kovách, A. G. B., Acta Physiol. Acad. Sci. Hung. **35**, 305 (1969).

16. Lieman, F., Naunyn-Schmiedebergs Arch. Pharmakol. **268**, 161 (1971).

17. Fitzgerald, R. S., Leitner, L.-M., and Liaubet, M.-J., Resp. Physiol. **6**, 395 (1969).

Received May 15, 1972. P.S.E.B.M., 1972, Vol. 141.