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Received November 16, 1964. P.S.E.B.M., 1965, v118.

Effect of Simultaneous Infusions of ACTH and Angiotensin II Upon Aldosterone Secretion of Hypophysectomized Nephrectomized Dogs.* (29973)

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Several studies have shown that acute infusions of angiotensin II stimulate aldosterone secretion in hypophysectomized nephrectomized dogs(1,2,3). The secretion rates achieved, however, are not usually as high as the rates observed in secondary hyperaldosteronism in the dog(4,5, and unpublished observations). This failure to achieve high secretion rates in hypophysectomized nephrectomized dogs has been attributed to the depletion of precursors necessary for aldosterone biosynthesis due to absence of ACTH. A previous study(6) showed that infusions of small doses of ACTH (2-50 mU) stimulated only cortisol and corticosterone secretion, while large doses (100-1000 mU) stimulated aldosterone secretion as well. Experiments with extracts of dog anterior pituitary gave results that were similar to those obtained with commercial ACTH. The present experiments were designed to determine whether small doses of ACTH would enhance the stimulating effect of angiotensin II upon aldosterone secretion in the dog.

Methods. Male mongrel dogs weighing 13-15 kg underwent a left nephrectomy one to two weeks prior to the experimental day. On the experimental day, each dog was hypophysectomized under pentobarbital anesthesia. The right kidney was removed and the right lumboadrenal vein cannulated for collection of adrenal vein blood. Each sample of adrenal vein blood was replaced, volume for volume, with blood from hypophysectomized nephrectomized dogs. One hour after hypophysectomy and nephrectomy, a constant infusion of either 0.042 (Dogs C and D, Table I) or 0.17 μg/min of synthetic angiotensin II was started. These doses were found in a previous study(6) to stimulate aldosterone secretion while having no significant effect on the secretion of corticosterone and 17-hydroxycorticoids. In 8 dogs that had been receiving angiotensin II by continuous infusion for one to 4 hours, the effect of a single injection of 5 mU of ACTH (Upjohn) upon adrenal secretion of 17-hydroxycorticoids, corticosterone and aldosterone was studied. In 4 dogs, the effect of a priming dose of 5 mU ACTH, followed by a constant infusion of ACTH (30 mU/hour) added to the constant infusion of angiotensin II (0.17 mμg/min) was studied. The ACTH infusion was started after the angiotensin II infusion had been running one hour and 40 minutes. A series of preliminary experiments indicated that this dose of ACTH did not increase aldosterone secretion by itself, yet stimulated 17-hydroxycorticoids and corticosterone significantly. Corticosterone, aldosterone and 17-hydroxycorticoid secretion were measured by methods previously described(1,6).

Results. In Table I are presented the results of single injections of 5 mU of ACTH upon adrenal secretions of hypophysectomized nephrectomized dogs receiving constant infusions of angiotensin II. The secretion rates shown are the rates measured immediately before and during the fourth to fourteenth minute after injection of ACTH. Despite a striking stimulation of 17-hydroxycorticoid and corticosterone secretion, there was no consistent change in aldosterone se-

* This work was supported by USPHS Grants AM 06704 and AM 05954.

TABLE I. Effect of Single 5 mU Injection of ACTH During Constant Angiotension II Infusion.

	Pre ACTH			Post ACTH			Change		
Dog	170H*	B*	Aldo†	170H*	B*	Aldo†	170H*	B*	Aldo†
A	0	.16	28	9.7	1.9	34	9.7	1.8	6
B	1.2	.20	19	.9	.88	13	−.3	.68	−6
C	.1	.033	3	3.6	1.2	2	3.5	1.2	−1
D	.3	.037	3	.6	2.2	1	.3	2.2	−2
E	.3	—	12	4.3	—	16	4.0	—	4
F	.3	.18	20	8.6	2.0	19	8.3	1.8	−1
G	0	.20	52	4.2	1.3	52	4.2	1.1	0
H	.9	.79	12	6.7	.99	4	5.8	.2	−8
							Mean = 5.1	1.3	−1

* μg/min. † Aldosterone mμg/min.

TABLE II. Effect of Addition of ACTH Infusion to Angiotensin II Infusion.

	Pre ACTH			Post ACTH			Change		
Dog	170H*	B*	Aldo†	170H*	B*	Aldo†	170H*	B*	Aldo†
E	.4	—	13	3.7	—	20	3.3	—	7
F	.3	.13	26	8.7	1.5	25	8.4	1.3	−1
G	.2	.18	51	4.3	1.8	69	4.1	1.7	18
H	1.2	.40	9	7.7	1.2	6	6.5	.83	−3
							Mean = 5.6	1.28	5

* μg/min. † Aldosterone mμg/min.

cretion. The effect of adding a constant infusion of ACTH to a constant infusion of angiotensin II is shown in Table II. Each of the secretion rates represent the mean of 3 to 4 collection periods taken at half-hour intervals. In only one dog was there a substantial increase in aldosterone secretion following addition of the ACTH infusion. In the other 3, there was little or no change despite significant and persistent increases in 17-hydroxycorticoid and corticosterone secretion.

Discussion. The present experiments demonstrated that the stimulation of the adrenal by ACTH during a constant infusion of angiotensin II did not enhance the aldosterone stimulating effect of the angiotensin II infusion. The dose of ACTH used stimulated 17-hydroxycorticoid and corticosterone secretion markedly but not aldosterone secretion. Corticosterone is a major precursor for aldosterone biosynthesis. Depletion of corticosterone or some other precursor controlled by ACTH, therefore, does not appear to be the explanation for the failure of angiotensin II infusion to raise the secretion rate of aldosterone in hypophysectomized nephrectomized dogs(1,2,3) to levels observed in dogs with secondary hyperaldosteronism(4,5). It is possible that acute hypophysectomy plus nephrectomy have a non-specific inhibitory effect upon aldosterone production. However, no such inhibition of the 17-hydroxycorticoid and corticosterone response to ACTH is observed; the secretion of these compounds attains the levels found in acutely stressed dogs with pituitaries and kidneys. An alternative explanation is that hyperplasia of the zona glomerulosa is necessary before secretion rates can reach the values observed in secondary hyperaldosteronism.

Summary. Addition of ACTH to constant infusions of angiotensin II in hypophysectomized nephrectomized dogs resulted in marked stimulation of 17-hydroxycorticoid and corticosterone secretion but no change in aldosterone secretion. Therefore, the absence of ACTH in hypophysectomized dogs does not explain the failure of acute angiotensin II infusions to raise aldosterone secretion to the levels observed in secondary hyperaldosteronism in the dog.

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Received November 23, 1964. P.S.E.B.M., 1965, v118.

Rapid Pulmonary Removal of 5-Hydroxytryptamine in the Intact Dog.* (29974)

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Non-platelet bound 5-hydroxytryptamine (5-HT; serotonin) rapidly disappears from the circulation after injection(1,2). Monoamine oxidase activity and platelet binding *in vivo* and chemical inactivation by erythrocytes *in vitro* may occur to decrease the concentration of 5-HT prior to assay. Metabolism of 5-HT in man is primarily due to oxidative deamination by monoamine oxidase (3), which is widely distributed and particularly rich in liver, kidney, heart, and intestinal mucosa(4).

Previous studies utilizing 5-HT infusion into the liver(5) or perfusion of the isolated lung(6,7) have shown that 5-HT is removed by the liver and lung. Certain physiologic responses, however, may be altered by perfusion of the isolated lung(8). It seemed advisable, therefore, to evaluate pulmonary 5-HT removal *in vivo,* and to assess the role of β-phenylisopropylhydrazine‡ (PIH), a potent monoamine oxidase inhibitor.

Methods and materials. Adult mongrel dogs weighing from 12-15 kg were anesthetized with sodium nembutal (25 mg/kg), and both carotid arteries were cannulated with #260 polyethylene cannulae. A #6 Cournand catheter was introduced into a jugular vein and advanced so that its tip lay in the pulmonary artery, as confirmed by the characteristic pressure contour.

5-HT was used as the 3′-C^{14} creatinine sulfate§ (specific activity 11.4 mc/mM), and was dissolved in indocyanine green dye (IG),‖ final concentration 1.25 mg/ml. Six dogs were studied without pretreatment, and 4 following intravenous pretreatment with 10 mg/kg of PIH, given 2 hours before 5-HT injection. Each dog was given 2 injections of C^{14} 5-HT at approximately 30-minute intervals, the second injection being designated by the prime in Tables I and II.

Three to five ml of IG were rapidly injected into the pulmonary artery without, and with, added C^{14} 5-HT. Continuous carotid arterial blood samples were obtained thereafter for radioactivity measurement. Sample tubes were changed every 2 seconds. Five mg of EDTA (per 3-5 ml blood) was used as an anticoagulant. After thorough mixing, a 0.5 ml aliquot was added to 1.5 ml distilled water. It was frozen and thawed twice, and 20 μl of hemolysate were plated on duplicate 1.25 inch aluminum planchets by the method of Smith *et al*(9). Radioactivity was counted on an end window automatic gas flow counter having a counting efficiency of 23%. Corrections were made for the resolving time of the counter, and samples were counted at infinite thinness. Samples were counted for a minimum of 1000 counts. Samples were corrected for background, and were counted to an error of 4% or less at the 96% confidence level. Certain samples were cen-

* Supported by grants from U.S.P.H.S. (H-7150 and HE-06329) and Am. Cancer Soc.

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