5. Cristofori, F. C., Duncan, G. G., Metabolism, 1964, v13, 303.

6. Henry, R. J., Sobel, C., Kim, J., Am. J. Clin. Path., 1957, v28, 152.

7. Kingsley, G. R., Schaffert, R. R., in Standard Methods of Clinical Chemistry, M. Reiner, Ed., Academic Press, Inc., New York, 1953, v1, p55.

8. Lieber, C. S., Davidson, C. S., Am. J. Med., 1962, v33, 319.

9. Yu, T. F., Sirota, J. H., Bergen, L., Halpern, M., Gutman, A. P., Proc. Soc. Exp. Biol. and Med., 1957, v96, 809.

10. Nichols, J., Miner, A. T., Jr., Hiatt, E. P., J. Appl. Physiol., 1951, v3, 501.

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

Effect of Dietary Sodium Restriction on Adrenal Cortical Response to ACTH.* (29972)

WILLIAM F. GANONG, A. T. BORYCZKA, R. SHACKLEFORD, R. M. CLARK AND R. P. CONVERSE

Department of Physiology, School of Medicine, San Francisco Medical Center, University of California, San Francisco

In dogs fed a commercial dog food providing a daily intake of about 40 mEq of sodium, the dose of ACTH required to increase aldosterone output is larger than the dose producing maximal glucocorticoid secretion (1,2). A low sodium diet stimulates aldosterone secretion, but does not increase glucocorticoid output(3,4), suggesting that ACTH plays no role in the adrenocortical response to sodium restriction. However, this conclusion assumes that the low sodium diet has no effect on the adrenocortical response to ACTH. The present studies demonstrate that although there is no change in the glucocorticoid response to ACTH, the aldosterone-stimulating activity of ACTH is increased in dogs fed a low sodium diet.

Materials and methods. Fourteen male mongrel dogs weighing 5.8 to 11.2 kg were subjected to left nephrectomy. They were then fed a diet providing less than 1 mEq of sodium and 20 mEq of potassium per day for 5 days (4 dogs), 14 days (6 dogs), or 2 months (4 dogs). The changes in aldosterone and 17-hydroxycorticoid secretion produced in these dogs by 2, 5, 10, 50 and 100 mU doses of ACTH were then measured. Each of the dogs was anesthetized with pentobarbital, the right kidney removed, the right lumboadrenal vein cannulated(5), and the dog hypophysectomized. One hour after hypophysectomy (2 hours in the case of dogs on the low sodium diet for 2 months), a control adrenal venous blood collection was made and the adrenocortical response to various doses of ACTH then tested. ACTH (Upjohn) was administered intravenously. Adrenal venous blood was collected from the 4th to 14th minute after injection. Thirty minutes after injection (60 minutes in the case of most of the 50 mU doses) another adrenal venous blood specimen was obtained, following which another dose of ACTH was injected. The 100 mU dose was injected last, and the animal sacrificed after the first adrenal venous blood collection following this dose. The samples were collected in centrifuge tubes containing 3 drops of heparin solution, centrifuged promptly, and the plasma frozen for subsequent determination of aldosterone content by the method of Kliman and Peterson(6), and 17-hydroxycorticoid content by the method of Silber and Porter(7). Output of these hormones was calculated by multiplying their concentration in the adrenal venous blood by the adrenal venous plasma flow per minute. Comparably prepared control dogs that had been fed a commercial diet providing about 40 mEq of sodium were injected with the same doses of ACTH; most of the results in these control dogs have been published previously(1).

In all dogs, blood pressure was monitored throughout the experiment using a Grass Model 5 polygraph and a Statham strain

* Supported by USPHS Grant AM06704.

TABLE I. Effect of Restricting Sodium Intake on Aldosterone-Stimulating Effect of Various Doses of ACTH in Nephrectomized Hypophysectomized Dogs. Values are means ± standard errors of outputs after ACTH minus control outputs. Numbers in parentheses are number of dogs.

ACTH dose (mμ)	Change in aldosterone output (mμg/min)			
		Low salt diet		
	Normal diet	5 days	14 days	2 mo
2	2 ± 3.2 (4)	1 ± 3.9 (3)	5 ± 7.2 (6)	25 ± 19.4 (4)
5	4 ± 1.8 (7)	7 ± 3.9 (4)	17 ± 9.3 (6)	47 ± 17.5 (4)
10	-3 ± 3.4 (6)	16 ± 8.8 (4)	22 ± 5.9 (6)	63 ± 67.6 (4)
50	4 ± 1.4 (3)	35 ± 10.1 (4)	52 ± 10.9 (6)	138 ± 54.9 (3)
100	27 ± 1.8 (3)	42 ± 11.0 (4)	48 ± 9.9 (5)	100 (2)
1000	49 ± 9.9 (10)			

TABLE II. Effect of Restricting Sodium Intake on 17-Hydroxycorticoid-Stimulating Effect of ACTH in Nephrectomized Hypophysectomized Dogs. See legend for Table I.

ACTH dose (mμ)	Change in 17-hydroxycorticoid output (μg/min)			
		Low salt diet		
	Normal diet	5 days	14 days	2 mo
2	2.3 ± .9 (4)	2.2 ± 1.1 (3)	2.5 ± .6 (6)	1.3 ± .3 (4)
5	3.5 ± .7 (8)	6.7 ± 2.2 (4)	4.9 ± .9 (6)	4.0 ± .8 (4)
10	7.9 ± 2.0 (5)	6.9 ± 2.1 (4)	7.0 ± .8 (6)	6.0 ± 1.2 (4)
50	7.5 ± 1.9 (3)	7.5 ± 1.9 (4)	7.8 ± .5 (5)	9.3 ± .3 (3)
100	8.6 ± 1.6 (3)	6.6 ± 2.1 (3)	8.0 ± .3 (5)	10.2 (2)
1000	8.5 ± .4 (10)			

gauge attached to a catheter in the femoral artery. All blood loss was replaced with bank blood from donor dogs. The low sodium diet was made up by mixing Lonalac® and sucrose with distilled water(8). At autopsy, the right adrenal glands were removed, cleaned and weighed.

Results. The mean changes in aldosterone and 17-hydroxycorticoid output following each dose of ACTH are summarized in Tables I and II. It is apparent that the aldosterone-stimulating activity of ACTH increased in dogs fed a low sodium diet, and that the magnitude of the increase was proportional to the length of time on the diet. There is considerable variability from dog to dog in the magnitude of this increase, but the aldosterone response to 50 mU of ACTH is significantly greater than that of the controls ($p<0.05$) after only 5 days of salt restriction, and the responses to 10 and 50 mU of ACTH are significantly greater ($p<0.01$ in both cases) after 14 days on the low sodium diet. In the case of the dogs on the low sodium diet for 2 months, 3 had very high aldosterone output values while one had only slightly elevated values; consequently, the standard errors of the means at this time were very large. There were no significant changes in 17-hydroxycorticoid outputs in any of the 3 groups of dogs.

Right adrenal weight in a previously reported series of 10 normal dogs in this laboratory averaged 54.7 ± 4.8 mg/kg(9). In the present experiments, the mean weights of the right adrenals of the dogs fed a low sodium diet for 5 days, 14 days, and 2 months were 85.1 ± 8.0 (standard error), 64.0 ± 6.1, and 73.5 ± 11.6 mg/kg respectively. The first of these values is significantly greater than normal, but the other 2 are not.

Blood pressure remained in the generally normal range throughout all the assays. Control 17-hydroxycorticoid outputs in each of the dogs were less than 14% of the maximum output produced by ACTH. This indicates that the hypophysectomies were functionally complete; we have found that 17-hydroxycorticoid output is the most sensitive indicator of the completeness of hypophysectomy (10), and that in dogs in which there are no pituitary remnants on microscopic examination of serial sections of the hypothalamus, 17-hydroxycorticoid output 1 hr after hypo-

physectomy averages 16.7% of maximum output, with a standard deviation of 8.2% (Ganong and Lowe, unpublished).

Discussion. An increase in the aldosterone-stimulating activity of ACTH associated with sodium depletion was first reported in humans(11-13), and it may also occur in sheep (14). The increase in dogs is so large that during prolonged sodium restriction, minor increases in ACTH secretion must contribute appreciably to the elevation in aldosterone secretion. Adrenals from rats fed a low sodium diet have been reported to produce more aldosterone *in vitro* than adrenals from control rats(15), although their response to graded doses of ACTH has apparently not been tested. The adrenals of salt depleted rats also have a decreased maximum corticosterone output(16,17), presumably because the hypertrophied zona glomerulosa encroaches on the zona fasciculata and zona reticularis. However, the present results show that reduced glucocorticoid output does not develop in the dog, and Dahl and his associates(18) found that it did not develop in humans.

The converse response, *i.e.*, a decrease in aldosterone-stimulating activity of ACTH in animals on a high salt diet, has not been reported. Preliminary results in our laboratory suggest that such inhibition does occur in the dog. There does not appear to have been a careful study of the effects of variations in sodium intake on adrenocortical response to angiotensin II.

The increases in adrenal sensitivity to the aldosterone-stimulating action of ACTH correlate temporally with hypertrophy of the zona glomerulosa in animals on a low sodium diet. This hypertrophy presumably accounts for the increased adrenal weight of our animals. In another series of dogs fed the low salt diet for 2 weeks, definite hypertrophy of the zona glomerulosa was found, and this hypertrophy was very marked in dogs fed the diet for 2 months (Clark and Converse, unpublished). The inner 2 zones of the adrenal cortex were normal in these animals. Hypertrophy of the zona fasciculata and zona reticularis is known to be associated with an increased adrenal sensitivity to the glucocorticoid-stimulating effects of ACTH(19). Presumably, therefore, the increases in the two situations are due to the operation of similar mechanisms, but the underlying biochemical explanation of the increases remains a mystery.

Summary. In dogs fed a low sodium diet for 5 days before being hypophysectomized and nephrectomized, ACTH exerts a greater aldosterone-stimulating effect than it does in dogs fed approximately 40 mEq of sodium/day. Longer periods of sodium restriction produce a further increase in this action of ACTH. Seventeen-hydroxycorticoid output in response to various ACTH doses is unaffected.

The authors wish to thank Dr. J. Stucki, Upjohn Co., Kalamazoo, Mich., for ACTH used in these studies, and Meade-Johnson Co., Evansville, Ind., for Lonalac used to make up the low sodium diets.

1. Mulrow, P. J., Ganong, W. F., Cera, G., Kuljian, A., J. Clin. Invest., 1962, v41, 505.

2. Ganong, W. F., Advances in Neuroendocrinology, U. Illinois Press, Urbana, 1963, 92.

3. Ulick, S., Laragh, J. H., Lieberman, S., Pr. Soc. Am. Physicians, 1958, v71, 225.

4. Luetscher, J. A., Jr., Recent Progress in Hormone Research, 1956, v12, 175.

5. Hume, D. M., Nelson, D. H., Surg. Forum, 1955, v5, 568.

6. Kliman, B., Peterson, R. E., J. Biol. Chem., 1960, v235, 1639.

7. Silber, R. H., Porter, C. C., ibid., 1954, v210, 923.

8. Ganong, W. F., Lee, T. C., Van Brunt, E. E., Biglieri, E. G., Endocrinology, 1965 in press.

9. Wise, B. L., Van Brunt, E. E., Ganong, W. F., Proc. Soc. Exp. Biol. and Med., 1964, v116, 306.

10. Ganong, W. F., Hume, D. M., Endocrinology, 1956, v59, 292.

11. Müller, A. F., Riondel, A. N., Manning, E. L., Helv. med. Acta, 1956, v23, 572.

12. Thorn, G. W., Ross, E. J., Crabbé, J., van't Hoff, W., Brit. Med. J., 1957, v2, 1955.

13. Venning, E. H., Dyrenfurth, I., Dosseter, J. B., Beck, J. C., Metabolism, 1962, v11, 254.

14. Blair-West, J., Coghlan, J. P., Denton, D. A., Coding, J. R., Wintour, M., Wright, R. D., Recent Progress in Hormone Research, 1963, v19, 311.

15. Eisenstein, A. B., Proc. Soc. Exp. Biol. and Med., 1959, v101, 850.

16. Eisenstein, A. B., Strack, I., Endocrinology, 1961, v68, 121.

17. Marx, A. J., Deane, H. W., Mowles, T. F.,

Sheppard, H., ibid., 1963, v73, 329.

18. Dahl, L. K., Silver, L., Sparagen, S., Smilay, M., Nature, 1961, v190, 348.

19. Ganong, W. F., Hume, D. M., Endocrinology, 1956, v59, 302.

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)

PATRICK J. MULROW AND WILLIAM F. GANONG

Department of Medicine, Yale University School of Medicine, New Haven, Conn. and Department of Physiology, University of California School of Medicine, San Francisco

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.