THE EFFECT OF POTASSIUM DEFICIENCY UPON ADRENOCORTICAL SECRETION IN THE RAT [1]

By RAY H. ROSENMAN, SHIRLEY ST. GEORGE, S. CHARLES FREED, AND MALCOLM K. SMITH

(From the Harold Brunn Institute, Mount Zion Hospital, San Francisco, Calif.)

(Submitted for publication July 11, 1955; accepted August 10, 1955)

The potassium-deficient rat exhibits several features in common with the untreated adrenalectomized rat, among which are subnormal growth, lowered blood pressure (1–3), diminished blood pressure responsiveness to pressor substances (4), and decreased tolerance to trauma. It therefore was considered important to determine whether the depressor response induced by potassium depletion might in fact be due to an associated state of adrenocortical insufficiency induced by the chronic potassium depletion. Such a mechanism appeared plausible particularly in view of our finding that the administration of cortisone rapidly returns the lowered blood pressures induced by potassium deficiency in rats to their initially normotensive (5) or hypertensive levels (6).

MATERIALS AND METHODS

A series of male rats (Long-Evans strain), initially aged 5 to 6 weeks, was used in this investigation. The rats were divided into three groups. One group of 6 rats (I) was fed a synthetic ration deficient in potassium, as previously described (2); this diet contained 0.004 per cent potassium and 1.4 per cent sodium.[2] A second group of 8 control rats (II) was fed the same synthetic ration but with added potassium chloride; it contained 0.52 per cent potassium and 1.4 per cent sodium.[2] For further control purposes a third group of 10 rats (III) was fed stock laboratory ration (containing 0.78 per cent potassium, 0.80 per cent sodium[2]). Seven weeks later the blood pressure of each rat was obtained with the microphonic manometer (7). Each rat was tail-bled for determination of plasma potassium 48 hours prior to collection of adrenal venous blood.

The secretion of adrenocortical steroids then was determined in all rats by the following technique. Under light ether anesthesia the rat was injected intramuscularly with 2 mg. sodium heparin, the abdominal cavity of the rat was opened and the right adrenal gland excised. The left kidney was "slipped" out of its capsule and removed after ligation of its vascular pedicle. A polyethylene cannula was introduced into the left renal vein through a small incision just proximal to the pedicle ligature and was ligated in place in such a manner that its bevelled distal tip approximated the left adrenal vein as the latter entered into the renal vein. Any accessory veins which emptied into the adrenal vein were then ligated. Another ligature was placed about the left renal vein at the juncture with the inferior vena cava. The cannula was brought out through a left lateral stab wound, the abdominal incision closed and the animal placed in a restraining cage. The adrenal venous blood was drained by gravity into a cold, heparinized test tube held in a chilled container filled with ice. In general, collection of blood was continued over a period of 60 to 120 minutes, during which 2.5 to 4.0 ml. of blood was obtained.

Each sample of adrenal venous blood was subjected to the following analysis for its steroid content. One to 2 ml. of plasma were extracted with 50 ml. of chloroform. The phenylhydrazine chromogen was extracted with 1 ml. reagent and developed overnight at room temperature according to the procedure of Silber and Porter (8). The curves of the reactive steroids were read between 320 and 450 mμ, using 0.5 ml. cuvettes in a Beckman Model DU spectrophotometer. From these data values were computed for steroids absorbing maximally at 350 and 410 mμ, by the system of Vickerstaff (9) for two component mixtures on the assumption (10, 11) that corticosterone (compound B) and 17-hydroxycorticosterone (compound F) are the main products of the adrenal effluent, other C_{19} and C_{21} steroids which have been detected by paper chromatography contributing less than 10 per cent of the total absorption at each of the two significant wave lengths, 350 and 410 mμ. The fact that for the rat, the extinction of 17,21-dihydroxy, 20-keto-steroids at 410 mμ is 5 times that of the predominating corticosterone at 350 mμ (8) and also that the absorption peaks are well separated, validates the use of this type of calculation. The amount of Silber-Porter chromogen of aldosterone is too small to be detected by this method, since aldosterone is secreted at a rate of only 0.2 to 0.8 μg per Kg. per hr. (11). Corticosterone (Merck)[3] was used as the standard for compounds with absorption maxima at 350 mμ and 17-hydroxycorticosterone (Merck) for those at 410 mμ. Most of the curves

[1] Aided by Grants from the American Heart Association, Life Insurance Medical Research Fund, San Francisco Heart Association and the National Institute of Health, Grant H-1006, Public Health Service.

[2] As determined by flame photometry of ashed diet.

[3] Provided by the courtesy of Dr. Frederick K. Heath of Merck & Co., Inc., Rahway, New Jersey.

TABLE I

Effect of potassium depletion on adrenocortical secretion of the rat

Group	Type of rat	Rat no.	Weight (gm.)	3-keto, Δ4, adrenal steroids calculated as corticosterone: Concentration (μg/ml. plasma)	3-keto, Δ4, adrenal steroids calculated as corticosterone: Secretory rate (μg/Kg./hour)	17,21-dihydroxy, 20-keto, steroids calculated as 17-hydroxycorticosterone: Concentration (μg/ml. plasma)	17,21-dihydroxy, 20-keto, steroids calculated as 17-hydroxycorticosterone: Secretory rate (μg/Kg./hour)
I	Rats fed potassium-deficient diet	12A	180	16.2	96.9	2.2	13.4
		13A	220	15.6	180.0	2.0	22.5
		14A	250	30.0	305.0	2.7	28.0
		15A	240	25.0	229.0	1.2	11.0
		16A	196	16.2	186.2	1.5	17.3
		17A	230	33.7	84.7	4.3	10.8
	Average: 6		219	22.7	180.3	2.3	17.1
	Range:			(15.6–33.7)	(84.7–305.0)	(1.2–4.3)	(10.8–28.0)
II	Rats fed potassium-deficient diet with added KCl	36A	250	12.5	217	0.7	12.1
		37A	250	13.7	303	1.8	39.4
		38A	242	16.2	92	1.0	5.7
		39A	242	23.6	369	2.0	3.1
		40A	236	23.7	182	1.8	14.0
		41A	200	23.6	184	1.2	9.3
		42A	218	13.7	217	0.7	10.0
		46	240	17.5	178	2.0	21.0
	Average: 8		235	18.0	215	1.4	14.3
	Range:			(12.5–23.7)	(92–369)	(0.7–2.0)	(3.1–39.4)
III	Rats fed stock diet	3A	200	57.5	290	5.0	25.0
		6A	218	15.3	135	1.3	11.6
		7A	298	16.2	164	1.5	15.2
		8A	230	11.2	64	0.8	4.7
		47	212	30.0	129.3	3.1	13.2
		48	252	14.8	114.8	0.8	6.4
		49	308	6.2	56.9	0.6	5.6
		50	252	26.2	216.4	1.5	13.4
		51	238	26.2	168	1.8	11.3
		2A	220	30.0	266	4.2	37.4
	Average: 10		238	23.3	160.4	2.0	14.2
	Range:			(6.2–57.5)	(56.9–290)	(0.6–5.0)	(4.7–37.4)

of the derived values reported showed absorption maxima at 350 mμ, rarely a shift to 380 and 390 mμ was noted.

RESULTS

The individual and average values for the steroids obtained from the three groups of rats are shown in Table I. At that time the average blood pressure of the potassium-deficient rats was 84 mm. Hg (Range: 78 to 88) in contrast with the average blood pressure of 108 mm. Hg (Range: 94 to 120) in the control rats fed stock diet. The average plasma K of the deficient rats was 3.5 mEq. per L. (Range: 2.7 to 3.7) and that of the control rats fed stock diet was 5.6 mEq. per L. (Range: 5.1 to 6.6). Elsewhere (3, 5, 12) we have shown that muscle potassium depletion also is present in such rats. The results are presented in terms of the concentrations (μg per ml. plasma) and rates of secretion (μg per Kg. weight per hour) of 3 keto, Δ4, adrenal corticosteroids calculated as corticosterone (compound B) and of 17,21,hydroxy, 20-keto-corticosteroids calculated as 17-hydroxycorticosterone (compound F).

As can be noted in Table I, the potassium-deficient rat as well as the normal animal is capable of putting out considerable amounts of steroids, the deficient animal averaging 180.3 μg per Kg. per hr. and the control rat, 160.4 μg per Kg. per hr. Since the concentrations of corticosterone in the plasma of the two groups are quite similar (22.7 and 23.3 μg per ml. plasma), this slight difference in secretory rates may be accounted for on the basis of size, the deficient animals being slightly smaller. In general, corticosterone was

found to be secreted in a tenfold greater concentration than was 17-hydroxycorticosterone. The concentrations and rates of secretion of 17-hydroxycorticosterone in the potassium-deficient rats were of the same magnitude as in the control rats.

It can be noted that considerable variation occurred in the plasma concentrations and rates of secretion of both steroids. This seems to be characteristic of the adrenal secretion of various animals tested by present day methods (10, 13–15). However, with only an occasional exception, the range of values was generally similar in all three groups of animals. Considering the relatively small sampling, the average values observed in the three groups also appeared to be of similar magnitudes.

DISCUSSION

Previous studies from this laboratory have shown that dietary potassium deprivation induces a fall of blood pressure in normotensive (1) and hypertensive (2, 3) rats which is a specific effect of potassium depletion (16) and which occurs in association with a somewhat diminished blood pressure responsiveness to pressor substances (4). Administration of potassium to such rats rapidly restores their blood pressure responsiveness and their blood pressures (16), if the adrenals are intact (12). We have considered (17) the possible role of insufficiency of adrenocortical secretion in this depressor response to potassium deficiency because of these and other common features of the potassium-deficient and the untreated, adrenalectomized rat. That potassium depletion might induce a hypotensive response by suppressing adrenocortical function also was suggested by the fact that potassium deficiency evokes a chronic alarm reaction (18) as well as by our earlier observation (5, 6) that cortisone promptly restores the lowered blood pressures of potassium-deficient rats to their respective normotensive or hypertensive levels without altering their potassium-depleted state.

On the other hand, some of the responses to potassium deficiency are inconsistent with a pattern of adrenocortical insufficiency. Thus, there is considerable indirect evidence that potassium deficiency stimulates production of adrenocorticoids (18–20) by evoking a chronic "alarm reaction" (18). Enlargement of the adrenals occurs despite inactivation of the glomerulosa zones, and the adrenals of such rats are depleted of ascorbic acid (18, 19, 21). Furthermore, such rats also exhibit increased liver glycogen and diminished glucose tolerance (18, 20), involution of the thymus (19, 20) and decreased circulating blood eosinophils and lymphocytes (18, 19). Although potassium deficiency appears to suppress the secretion of aldosterone (11), on the basis of present evidence it seems unlikely that the depressor response might be ascribed to this, since administration of the analogous hormone, DCA, is without pressor effect in potassium-deficient rats and indeed has a depressor and toxic effect in such animals (22).

We have attempted to clarify the relationship of potassium deficiency and adrenocortical function by direct determination of adrenal secretion in potassium-deficient rats. The values recorded for our control rats are in good agreement with those recorded by Bush (10) and by Singer and Stack-Dunne (11), despite the use of a completely different method of analysis. The data in this study indicate essentially similar averages and ranges of concentration and rates of secretion of corticosterone and 17-hydroxycorticosterone by the potassium-deficient rats, when compared to the control animals. It is important to point out that the technique employed for collection of adrenal venous blood in this study probably constitutes a maximal stimulus for adrenocortical secretion. Under these circumstances, however, the potassium-deficient hypotensive rat appeared to respond in a fashion similar to that of the control animals. It is of interest that Singer and Stack-Dunne (11) also recently found that potassium deficiency fails to affect corticosterone secretion in rats. Therefore, it must tentatively be suggested that the hypotensive response induced by potassium deficiency is not mediated by supression of adrenocortical secretion. This conclusion appears justified in view of the present data and the fact that trauma comparable to the operative procedure used here fails to raise the lowered blood pressure of the potassium-deficient rat.

SUMMARY

The effect of chronic potassium deficiency upon adrenocortical steroid secretion was studied in

rats. The data indicate that, under the stress of the experimental method, the rates of secretion of corticosterone and 17-hydroxycorticosterone by potassium-deficient rats are essentially similar to those of control animals. It is concluded that the hypotensive response induced by potassium depletion is not mediated by a suppression of adrenocortical secretion of these steroids.

ACKNOWLEDGMENT

We wish to express our appreciation to Hoffmann-La Roche Inc. for their generous supplies of Litrison, used to provide the vitamin supplement of the synthetic diets.

REFERENCES

1. Freed, S. C., and Friedman, M., Depressor effect of potassium restriction on blood pressure of the rat. Proc. Soc. Exper. Biol. & Med., 1951, **78**, 74.
2. Friedman, M., Rosenman, R. H., and Freed, S. C., Depressor effect of potassium deprivation on the blood pressure of hypertensive rats. Am. J. Physiol., 1951, **167**, 457.
3. Rosenman, R. H., Freed, S. C., St. George, S., and Smith, M. K., Effect of varying dietary potassium upon the blood pressure of hypertensive rats. Am. J. Physiol., 1953, **175**, 386.
4. Rosenman, R. H., Freed, S. C., and Friedman, M., The peripheral vascular reactivity of potassium-deficient rats. Circulation, 1952, **5**, 412.
5. Freed, S. C., Rosenman, R. H., St. George, S., and Smith, M. K., Effect of cortisone and ACTH on blood pressure of hypotensive potassium-deficient rats. Circ. Res., 1954, **2**, 41.
6. Rosenman, R. H., Freed, S. C., and Smith, M. K., Effect of cortisone on blood pressure of hypertensive rats deprived of dietary potassium. Am. J. Physiol., 1954, **177**, 325.
7. Friedman, M., and Freed, S. C., Microphonic manometer for indirect determination of systolic blood pressure in the rat. Proc. Soc. Exper. Biol. & Med., 1949, **70**, 670.
8. Silber, R. H., and Porter, C. C., The determination of 17,21-dihydroxy-20-ketosteroids in urine and plasma. J. Biol. Chem., 1954, **210**, 923.
9. Vickerstaff, T., The Physical Chemistry of Dyeing, 2nd ed., London, Interscience, 1954.
10. Bush, I. E., Species differences in adrenocortical secretion. J. Endocrinol., 1953, **9**, 95.
11. Singer, B., and Stack-Dunne, M. P., The secretion of aldosterone and corticosterone by the rat adrenal. J. Endocrinol., 1955, **12**, 130.
12. Freed, S. C., Rosenman, R. H., and Smith, M. K., Effect of adrenalectomy upon the pressor action of potassium in hypotensive, potassium-deficient rats. Circ. Res., 1954, **2**, 494.
13. Zaffaroni, A., and Burton, R. B., Corticosteroids present in adrenal vein blood of dogs. Arch. Biochem. & Biophys., 1953, **42**, 1.
14. Kass, E. H., Hechter, O., Macchi, I. A., and Mou, T. W., Changes in patterns of secretion of corticosteroids in rabbits after prolonged treatment with ACTH. Proc. Soc. Exper. Biol. & Med., 1954, **85**, 583.
15. Farrel, G. L., Steroids in adrenal venous blood of the dog: Venous-arterial differences across the adrenal. Proc. Soc. Exper. Biol. & Med., 1954, **86**, 338.
16. Friedman, M., Freed, S. C., and Rosenman, R. H., Effect of potassium administration on (1) peripheral vascular reactivity and (2) blood pressure of the potassium-deficient rat. Circulation, 1952, **5**, 415.
17. Freed, S. C., Rosenman, R. H., and Friedman, M., The relationship of potassium in the regulation of blood pressure with special attention to corticosteroid hypertension. N. Y. Acad. Sc. Conference of October 3–4, 1952. Ann. New York Acad. Sc., 1953, **56**, 637.
18. Gardner, L. I., Talbot, N. B., Cook, C. D., Berman, H., and Uribe, R. C., The effect of potassium deficiency on carbohydrate metabolism. J. Lab. & Clin. Med., 1950, **35**, 592.
19. Danford, H. G., and Herrin, R. C., Some physiological effects of salt restriction in the rat. Am. J. Physiol., 1951, **165**, 128.
20. Fuhrman, F. A., Glycogen, glucose tolerance, and tissue metabolism in potassium-deficient rats. Am. J. Physiol., 1951, **167**, 314.
21. Deane, H. W., Shaw, J. H., and Greep, R. O., The effect of altered sodium or potassium intake on the width and cytochemistry of the zona glomerulosa of the rat's adrenal cortex. Endocrinology, 1948, **43**, 133.
22. Rosenman, R. H., Freed, S. C., and Friedman, M., Effect of desoxycorticosterone acetate upon the blood pressure of rats fed varied dietary intakes of potassium and sodium. J. Clin. Endocrinol. & Metab., 1954, **14**, 661.