Proceedings
of the
Society
for
Experimental Biology and Medicine

VOL. 109 APRIL, 1962 No. 4

SECTION MEETINGS

CLEVELAND, O.
University Hospitals February 19, 1962

Effect of Cortisone and Spirolactone on Potassium Excreting Action of Aldosterone and Desoxycorticosterone.* (27329)

TETSUO UETE† AND ELEANOR H. VENNING (Introduced by J. S. L. Browne)
Department of Investigative Medicine, McGill University, and McGill University Clinic, Royal Victoria Hospital, Montreal, Canada

Previous investigations have shown that administration of aldosterone to adrenalectomized rats produced a more marked effect on urinary sodium excretion than on excretion of potassium or water(1,2). Similar observations have been made in adrenalectomized dogs with infusion of aldosterone into the renal artery, while in intact dogs there was an increased excretion of potassium with no demonstrable effect on sodium(3). Several studies have indicated that this action of aldosterone can be modified by simultaneous administration of hydrocortisone-like steroids both in man(4) and in adrenalectomized rats(5,6).

The present investigations were undertaken to obtain further information regarding the role of the glucocorticoids in modifying the action of aldosterone on excretion of electrolytes.

Method. Adrenalectomized mature male hooded rats, weighing 170 to 180 g were used in all experiments. The method of bioassay has been described previously in detail (5). The studies were performed with the following steroids: d-l-aldosterone, desoxycorticosterone-acetate (DCA), cortisone, and spirolactone (SC-11927). In each experiment, the effect of these steroids on water, sodium, and potassium excretion was studied separately or in combination with one another. Urine was collected over a 6-hour period after the simultaneous administration of 3.5 ml of water intraperitoneally and the test substances subcutaneously.

The sodium and potassium content of the urine was measured by flame photometry using an Internal Lithium Standard.

Results. The effects of increasing doses of d-l-aldosterone and desoxycorticosterone-acetate on excretion of water, sodium, and potassium are presented in Table I. Under the

* This work was supported by grants from Medical Research Council of Canada, the Cooper Fund of the Faculty of Medicine, McGill University, and the Banting Research Foundation to Dr. T. Uete.

† Research Fellow of Medical Research Council of Canada.

IN MEMORIAM

ABRAHAM J. GOLDFORB

1881 - 1962

Secretary-Treasurer, Society for Experimental Biology and Medicine

and

Managing Editor of the Proceedings

1924 - 1960

TABLE I. Effect of d-l-Aldosterone and Desoxycorticosterone-Acetate on Water and Electrolyte Excretion in Adrenalectomized Rats.

12 rats in each group	Urine ml/ 6 hr/rat	Na meq/6 hr/rat		K meq/6 hr/rat	
		Total excretion	Decrease in excretion	Total excretion	Increase in excretion
	Mean ± S.D.	Mean ± S.D.		Mean ± S.D.	
Exp. 1					
1. Control	4.4 ± 1.5	.294 ± .075	0	.131 ± .045	0
2. Aldosterone .02 μg	4.2 ± .8	.257 ± .050	.037	.150 ± .026	.019
3. " .10 "	3.4 ± .7	.201 ± .030	.093	.143 ± .025	.012
4. " .20 "	4.0 ± 1.7	.176 ± .071	.118	.156 ± .029	.025
5. " 1.00 "	3.8 ± .8	.103 ± .022	.191	.186 ± .087	.055
6. " 2.00 "	3.0 ± .8	.081 ± .031	.213	.190 ± .062	.059
Exp. 2					
1. Control	4.8 ± 2.4	.267 ± .007	0	.193 ± .027	0
2. DCA 1.0 μg	3.1 ± .9	.164 ± .017	.103	.198 ± .035	.005
3. " 3.0 "	2.7 ± .7	.104 ± .022	.163	.198 ± .057	.005
4. " 5.0 "	2.7 ± .7	.085 ± .021	.182	.222 ± .031	.029
5. " 12.0 "	2.7 ± 1.1	.039 ± .007	.228	.232 ± .064	.039

conditions of this assay in adrenalectomized rats, d-l-aldosterone in doses of 0.02 to 2.0 μg (Exp. 1) and DCA in doses of 1.0 to 12.0 μg (Exp. 2) caused a progressive retention of sodium while potassium was only slightly increased. Both aldosterone and DCA exerted a 2- to 7-fold greater effect on sodium than on potassium.

The effects of 0.5 μg of d-l-aldosterone and 100 and 200 μg of cortisone injected separately and combined are shown in Table II. In Exp. 3, aldosterone at this dose level caused a marked sodium retention, while 100 and 200 μg of cortisone increased the excretion of both sodium and potassium. When aldosterone and cortisone were administered together, the sodium retaining effect of aldosterone was lessened (–0.033 and –0.022 meq compared with –0.123 meq). The increase of potassium excretion by these 2 types of steroids given together was greater than the combined effects of these 2 steroids injected separately. When given together potassium rose by 0.097 and 0.121 meq and when given separately the sum of the two effects was 0.036 and 0.074 meq. The differ-

TABLE II. Effect of d-l-Aldosterone, Cortisone and Spirolactone on Water and Electrolyte Excretion in Adrenalectomized Rats.

12 rats in each group	Urine ml/6 hr/rat		Na meq/6 hr/rat		K meq/6 hr/rat	
	Total excretion	Change in excretion	Total excretion	Change in excretion	Total excretion	Change in excretion
	Mean ± S.D.		Mean ± S.D.		Mean ± S.D.	
Exp. 3						
1. Control	3.8 ± 1.0	0	.208 ± .054	0	.143 ± .034	0
2. Aldosterone .5 μg	4.0 ± 1.3	+ .2	.085 ± .011	—.123	.145 ± .034	+ .002
3. Cortisone 100 μg	5.3 ± .6	+ 1.5	.279 ± .082	+ .071	.177 ± .031	+ .034
4. Cortisone 200 μg	6.6 ± .9	+ 2.8	.350 ± .073	+ .142	.215 ± .008	+ .072
5. Aldosterone .5 μg Cortisone 100 μg	4.9 ± .6	+ 1.1	.175 ± .058	—.033	.240 ± .032	+ .097
6. Aldosterone .5 μg Cortisone 200 μg	4.9 ± 1.3	+ 1.1	.186 ± .061	—.022	.264 ± .036	+ .121
Exp. 4						
1. Control	4.0 ± 1.0	0	.125 ± .036	0	.138 ± .026	0
2. Aldosterone .5 μg	3.3 ± .8	— .7	.048 ± .012	—.077	.164 ± .019	+ .026
3. Cortisone 200 "	4.4 ± .9	+ .4	.168 ± .030	+ .043	.204 ± .033	+ .066
4. Spirolactone 500 μg	3.0 ± .4	— 1.0	.088 ± .021	—.037	.135 ± .034	—.003
5. Aldosterone .5 μg Cortisone 200 μg	5.4 ± .9	+ 1.4	.116 ± .049	—.009	.304 ± .046	+ .166
6. Aldosterone .5 μg Cortisone 200 μg Spirolactone 500 μg	5.8 ± .8	+ 1.8	.127 ± .031	+ .002	.227 ± .014	+ .089

ences in both the sodium and potassium results were statistically significant and p was <0.01 in each case.

In Exp. 4, administration of 500 μg of spirolactone caused a slight sodium retention but had no effect on potassium excretion. When this amount of spirolactone was injected simultaneously with 200 μg of cortisone and 0.5 μg of aldosterone, the increased potassium excretion by the concomitant use of cortisone and aldosterone was significantly lessened from + 0.166 to + 0.089 meq. The difference was statistically significant and p was <0.01.

Discussion. A more marked effect of aldosterone and DCA on reduction of sodium excretion than on increase in potassium excretion in the present investigation confirms the observations of other investigators in adrenalectomized animals(1,2) and in patients with Addison's disease(7,8). Sonnenblick *et al.*(9) demonstrated in man that administration of aldosterone resulted in significant chloride retention as well as sodium retention, and that the magnitude of the kaliuresis was less than the sodium retention. This evidence indicates that aldosterone acts by promoting sodium reabsorption in conjunction with chloride in addition to increasing cation exchange of sodium for potassium or hydrogen. The above findings together with the present results suggest that the greater effect of aldosterone on sodium retention than on potassium excretion may, therefore, be explained on the basis of the following mechanisms: aldosterone causes marked sodium and chloride reabsorption in the distal tubules and, possibly, in the proximal tubules independently of the potassium for sodium ion exchange process, or aldosterone increases the distal exchange of sodium for hydrogen ion with insufficient intraluminal sodium content at the potassium exchange site, and further the proportion of sodium exchanged with hydrogen ion distally may have been increased significantly.

When cortisone was injected simultaneously with aldosterone, potassium excretion was greater than the combined effects on this electrolyte when the steroids were administered separately, indicating a synergistic action of these 2 types of steroids on potassium excretion. We have obtained similar findings with corticosterone, hydrocortisone, and triamcinolone. As spirolactone does not alter the glomerular filtration rate(10), and is effective in diminishing this synergistic effect, the increased potassium excretion may well be related to secretion of this electrolyte in the distal tubules. According to Berliner (11), potassium excretion in the distal tubules is dependent upon the supply of sodium available for exchange. It has also been demonstrated, using mineralocorticoids in adrenalectomized animals, that potassium excretion is directly proportional to sodium load until a potassium excretory plateau is reached (12). Enhancement of the potassium excreting properties of aldosterone by cortisone-like steroids may be due to the fact that cortisone-like steroids increase the filtered load of sodium related to increased GFR(13,14). Consequently, an increased amount of intraluminal sodium would be available for exchange with potassium. On the other hand, cortisone-like steroids may play a specific role potentiating the potassium excreting activity of aldosterone in the distal tubules. There may be an additional transport mechanism for potassium independent of sodium.

Summary. In adrenalectomized rats, aldosterone and DCA exerted a greater effect on sodium retention than on potassium excretion or urine volume. Cortisone administered concomitantly with aldosterone enhanced the potassium excreting activity of aldosterone, and decreased the sodium retaining effect of aldosterone. The synergistic effect of these two types of steroids on potassium excretion was significantly diminished by spirolactone, suggesting that this increase in potassium excretion is related to secretion of this cation in the distal tubules.

The authors are greatly indebted to Prof. J. S. L. Browne and Dr. J. Dossetor for their interest in and criticism of this work, and to Dr. J. K. McGowan, C. D. Searle and Co., for spirolactone SC-11927.

1. Desaulles, P., Tripod, J., Schuler, W., *Schweiz. Med. Wschr.*, 1953, v83, 1088.

2. Sala, G., Luetcher, J. A., Jr., *Endocrinology*, 1954, v55, 516.

3. Barger, A. G., Berlin, R. D., Tulenko, J. F., *ibid.*, 1958, v62, 804.

4. Ross, E. J., *J. Clin. Endocrinol. and Metab.*, 1960, v20, 229.

5. Uete, T., Venning, E. H., *Endocrinology*, 1960, v67, 62.

6. Bailey, R. E., *ibid.*, 1960, v67, 807.

7. Dingman, J. F., Finkensteadt, J. T., Laidlaw, J. C., Renold, A. E., Jenkins, D., Merrill, J. P., Thorn, G. W., *Metabolism*, 1958, v7, 608.

8. Ross, E. J., Reddy, W. J., Rivera, A., Thorn, G. W., *J. Clin. Endocrinol. and Metab.*, 1959, v19, 289.

9. Sonnenblick, E. H., Cannon, P. J., Laragh, J. H., *J. Clin. Invest.*, 1961, v40, 903.

10. Wiggins, R. A., Hutchison, M. E., Carbone, J. V., Doolan, P. D., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 625.

11. Berliner, R. W., *Circulation*, 1960, v21, 892.

12. Cade, R., Shalhoub, R., *Abstract of 53rd Annual Meeting, Am. Soc. for Clin. Invest.*, 1961, p11.

13. Renzi, A. A., Renzi, M., Chart, J. J., Gaunt, R., *Acta Endocrinol.*, 1956, v21, 47.

14. Peters, G., *Arch. f. Exp. Path. und Pharmak.*, 1959, v235, 312.

Received January 15, 1962. P.S.E.B.M., 1962, v109.

Gamma-Ray Sensitivity of Oocytes of Immature Mice. (27330)

E. F. OAKBERG (Introduced by A. Hollaender)

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.*

The high radiation sensitivity of oocytes in early stages of follicular development in the adult female has been known since the 1907 report by Bergonié and Tribondeau(1) on ovarian changes in irradiated rabbits. These early observations have been confirmed histologically in both the mouse(2-7) and rat(8). The reduced fertility of irradiated female mice likewise indicates high sensitivity of early oocytes, since 1.4 litters are obtained after 300 r owing to high resistance of late follicle stages, yet killing of early oocyte stages with 50 r reduces the number of litters to 4 as compared to the 13 or 14 obtained from controls(9). Fractionation of radiation doses at high intensity, and especially reduction of the dose rate, markedly reduces the effectiveness of a given total dose as regards effects on the fertility of irradiated adult females(9).

All of the above results were obtained with adult mice. Russell *et al.*(10) found recently that the effect on fertility is much greater in immature mice of certain ages, even at low dose rates. Thus, whereas 85 r given at a rate of 90 r/week had no detectable effect on fertility of adult mice, the same dose given during the second week after birth induced permanent sterility either after a single litter or after a second litter of reduced size. Possible explanations of these results are: (*a*) newly formed dictyate oocytes are more sensitive to radiation than are comparable oocyte stages in the adult, (*b*) radiation sensitivity in young and adult mice is the same for equivalent oocyte stages, but fertility is less disturbed in the adult owing to the presence of more oocytes in later, more resistant stages of follicular development, (*c*) dose rate is unimportant in young mice, chronic and acute irradiation being equally effective, and (*d*) oocytes in young mice pass through an extremely sensitive stage of short duration, chronic irradiation therefore being highly effective since all young oocytes are exposed in this sensitive period. The experiments reported here indicate that early oocyte stages in 10-day-old mice are extremely sensitive to acute gamma-ray exposure, and that they are more sensitive to chronic irradiation than are similar stages in the adult.

Materials and methods. Pair matings of 101 strain females with C3H strain males were checked each morning, and any new litters recorded. When 9 days old, females were toe-marked, and assigned to the different dose groups, with only one mouse per litter being used for any treatment. Mice were irradiated between 10:00 and 11:05 a.m. on the tenth day after birth (day of birth being

* Operated by Union Carbide Corp., for U. S. Atomic Energy Commission.