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Determination of Antialdosterone Activity in Normal Dogs. (29977)

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Compounds having aldosterone-antagonistic activity are generally screened and evaluated in the laboratory by their ability to reverse the depressed urinary Na/K ratio of adrenalectomized rats given desoxycorticosterone acetate (DCA)(1). This report describes a method for detecting and evaluating aldosterone antagonists in a second laboratory species. The test is based upon the ability of an antagonist to reverse the depressed urinary Na/K ratio seen after administration of 9 α-fluorohydrocortisone (9FHC), a potent mineralocorticoid, to intact unanesthetized dogs.

Materials and methods. A colony of 15 trained, unanesthetized female hound dogs maintained at constant weight (18-25 kg) on Purina Lab Chow were used in all these experiments. The dogs were fed at 4:00 to 4:15 p.m. every afternoon. Water was allowed *ad libitum* except during the experiment. The dogs were housed in individual cages in a room having automatic constant control over temperature, humidity, and artificial lighting. Generally, 10 to 12 dogs were used on one day for each dose of compound. On the control and test days the animals were catheterized with a urinary retention catheter. The end of the catheter was clamped with a pinchcock and fixed around the dog's body with string, allowing subsequent urine collections without recatheterization. The bladder was emptied and, on test days, the animals were given an intramuscular injection of 9FHC in 0.25 ml of corn oil. The compound to be tested was then immediately given orally by means of a stomach tube in a small volume of water. Urine was collected and the total volume measured every 2 hours over a 6-hour period. Sodium and potassium were determined with a Baird Atomic Model KY-1 flame photometer adapted for automatic flow type analysis. Chloride was determined with a Technicon autoanalyzer.

Despite controlled experimental conditions, the normal Na/K ratio of the colony showed considerable variation when measured periodically over a period of one year. The least variation occurred when the Na/K ratio was determined on 2 successive days (Table I, control row). Therefore, for all subsequent tests a control 6-hour urine collection in which nothing was given to the animals was always obtained the preceding day. This control Na/K ratio was then used for statistical comparison with test day results.

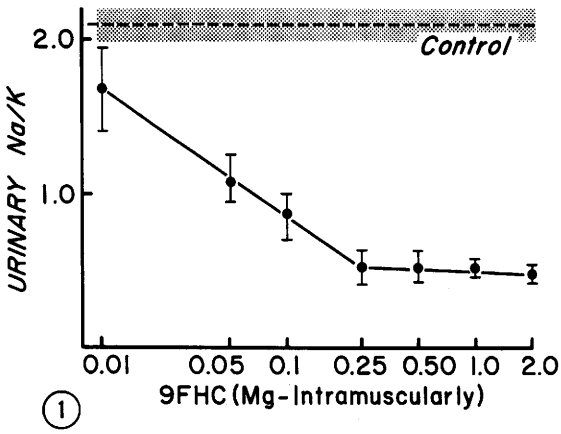
By subtracting the total volume and electrolyte excretion of the control from that of the test day, we were able to establish the level of activity exhibited by spironolactone (Aldactone). This compound has been clinically useful for reducing edema in states characterized by increased aldosterone production(3). In the present tests, we used a commercially available tablet form (Aldactone-A)(4) having a smaller particle size and reportedly better oral absorption.

Results. In order to evaluate the effect of an antagonist, the concentration of agonist is of primary importance(2). We therefore first determined the minimum dose of 9FHC necessary to cause the maximum decrease in the 6-hour urinary Na/K ratio of the dog. This titration is shown in Fig. 1. It is evident

TABLE I. Effect of Spironolactone-A and 9α-Fluorohydrocortisone (9FHC) on Electrolyte Excretion in Unanesthetized Dogs.

No. dogs	Mean 6-hr change (test day minus control day)				Na/K ratio	
	Urine vol	Na^+	K^+	Cl^-	Control	Test
	(ml)		(milliequivalents)			
			Control			
16	−10.5 ± 5.8	−.9 ± 1.3	−.58 ± .50	.9 ± 1.6	2.34 ± .59	2.58 ± .69
			.25 mg 9FHC			
10	−16.5 ± 37	−7.9 ± 1.9*	−.13 ± 1.3	−7.1 ± 4.9	1.62 ± .33	.53 ± .10*
			.25 mg 9FHC + spironolactone 3.0 mg/kg			
10	−21 ± 22	−.28 ± 2.6	.38 ± 1.6	1.9 ± 4.5	2.36 ± .52	2.17 ± .3
			.25 mg 9FHC + spironolactone 6.0 mg/kg			
10	−28 ± 51	2.2 ± 2.9	−1.3 ± 1.9	−.6 ± 4.8	1.68 ± .38	2.35 ± .58
			.25 mg 9FHC + spironolactone 12.0 mg/kg			
10	34.2 ± 29	6.2 ± 2.0*	−.6 ± 1.2	3.8 ± 2.7	1.48 ± .45	2.49 ± .44*
			Spironolactone 12.0 mg/kg			
5	4.8 ± 5.9	2.8 ± 1.6	−.6 ± .9	3.1 ± 2.1	1.76 ± .62	3.16 ± 1.19

* Significant difference ($p < .05$) between test and control data according to paired comparison t test (7). All tabulated values shown are means ± standard errors.



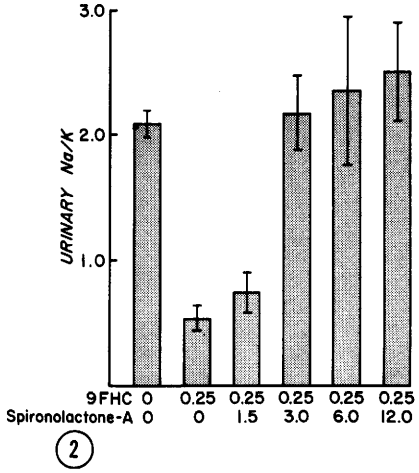


FIG. 1. Effect of 9α-fluorohydrocortisone (9FHC) on urinary Na/K of unanesthetized dogs. Control data represent normal urinary Na/K ratio of 141 determinations. Each dosage point represents means and standard errors of 6 to 12 determinations, all based on 6-hr urine collections from dogs of the same colony of 15.

FIG. 2. Effect of spironolactone-A (mg/kg orally) and 9α-fluorohydrocortisone (mg intramuscularly) on urinary Na/K ratio of unanesthetized dogs. Control data (no drugs) represent normal urinary Na/K ratio of 141 determinations. Each drug dosage point represents the means and standard errors of 10 determinations, all based on 6-hr urine collections from dogs of the same colony of 15.

from this curve that the dog is very sensitive to 9FHC. A total intramuscular dose of 0.25 mg causes a maximal depression of the urinary Na/K ratio, larger doses having no greater effect. The depression is based primarily on changes in sodium excretion (Table I). The 0.25 mg dose, therefore, was selected as the agonist dose.

Fig. 2 shows the effect of single oral doses of 1.5, 3.0, 6.0, and 12.0 mg/kg spironolactone-A on the depressed urinary Na/K ratio of dogs given 0.25 mg 9FHC. Spironolactone-A at doses of 3.0 and 6.0 mg/kg caused a reversal and a return to normal of the depressed urinary Na/K ratio, while a dose of 12.0 mg/kg caused a statistically significant increase over controls of the previous day.

Table I shows the total electrolyte excretory pattern of these same experiments. These results are presented as a difference in mean volume and electrolyte levels found in the 6-hour urine samples obtained on control and test days. Not shown in the table are the means and standard errors for control data of the 61 experiments, which for volume (ml), sodium, potassium, and chloride (mEq) per 6 hours were, respectfully, 107 ± 0.82, 9.37 ± 0.82, 7.10 ± 0.63, and 18.0 ± 1.68. The control row shows that there was no significant difference in urinary volume, electrolyte excretion, or Na/K ratio when urine was collected on 2 successive days. The dose of 0.25 mg 9FHC caused significant sodium retention, resulting in a significant decrease in the Na/K ratio. Addition to 9FHC of spironolactone-A in oral doses of 3.0 or 6.0 mg/kg caused an increase in sodium excretion and a return of the Na/K ratio to normal; however, there was no significant diuretic or natriuretic effect. With an oral dose of 12.0 mg/kg, however, there was a significant natriuresis, and urine volume increased, but not significantly so. The Na/K ratio was significantly increased above control values. Finally, spironolactone-A given in the absence of the mineralocorticoid 9FHC did not cause a significant diuresis or natriuresis at a dose of 12.0 mg/kg.

Discussion. The detection and assay of aldosterone antagonists in man has been described by Noel and Leahy(4) and by Ross (5). In these tests, 9FHC was given orally as the mineralocorticoid antagonist. The optimum oral total dose of 9FHC in the human in these studies was 1.0 mg, while in our experiments on the dog, an intramuscular total dose of 0.25 mg gave maximum urinary Na/K depression. Though the route of administration of 9FHC differed in these studies, the sensitivity of dog and man appears to be quite similar. In humans, the maximal activity of spironolactone is reached after 48 hours(4,5), so that the compound is administered in divided doses for 3 to 5 successive days before challenge with 9FHC. In dogs, however, it appears that activity of spironolactone-A can be seen in a single dose over a 6-hour period. The minimal dose of spironolactone-A that could be detected in the dog by a reversal of the depressed urinary Na/K ratio was 3.0 mg/kg (about 50 to 75 mg total dose). This is approximately the same as the total dose used in humans by Gantt and Dyniewicz(6) when spironolactone-A was given over a 3-day pretreatment period. Thus, the test in dogs appears to be as sensitive as that described in man and can discern doses of spironolactone-A in the human clinical dosage range (about 100 mg/day).

Spironolactone has been described as a specific competitive inhibitor of mineralocorticoids, since it is effective only in their presence (2). The testing procedure described here substantiates this by showing that administration of spironolactone-A alone at a dose of 12.0 mg/kg does not cause sodium loss, while this same dose in the presence of the potent mineralocorticoid 9FHC significantly increases sodium excretion (Table I).

Summary. A method is described for detecting an aldosterone antagonist over a 6-hour period in the unanesthetized intact dog. The method is based upon the ability of an aldosterone antagonist to overcome the depressed urinary Na/K ratio caused by administration of the potent mineralocorticoid 9 α-fluorohydrocortisone. Using this procedure, it was shown that spironolactone-A is active at an oral dose as low as 3 mg/kg, and at 12 mg/kg has a significant natriuretic effect.

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Experimental Analysis of the Rheoencephalogram (REG).* (29978)

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The method of measuring blood flow by electrical impedance plethysmography has been investigated since 1907(1), and its development has tended to parallel developments in the electronics field(2,3,4). All plethysmographic systems for blood flow measurements are limited by their inability to detect non-pulsatile blood flow. If the heart were a continuous pump, there would be no volume change associated with blood flow. However, the hollow heart muscle with its valves, produces a highly pulsatile flow and pulse flow information for any part of the body can be recorded.

Polzer and Schuhfried(5) passed an alternating current of 20 kilocycle through electrodes on the head of 2 subjects and recorded pulsatile fluctuations of electrical conductivity. They also noted diminished amplitudes in a patient with carotid occlusion. With the recent interest in cranial plethysmography or rheoencephalography it has become important to prove the validity of assumptions used to study clinical abnormalities of cerebral blood flow. This latter situation is particularly challenging because the skull is a rigid container without measurable plethysmographic change associated with blood flow.

Methods. For analysis of hemodynamic factors influencing the rheoencephalogram, or REG, an artificial perfusion system was chosen which made the control of stroke volume, pulse pressure and pulse rate possible. Greyhounds weighing about 25 kg were anesthetized with sodium thiobarbital and injected intravenously with 100 mg heparin. Venous blood from the right atrium was drained *via* a jugular catheter into a pump-oxygenator system and returned under pressure into both common carotid arteries (Fig. 1). Stroke volume and rate of an elastic ventricle pump with ball valves could be regulated by compressed air(6). Before entering the common carotid arteries, blood flow was measured with an electro-magnetic flowmeter(7) and blood pressure with a strain gauge. Lead foil electrodes with silastic insulation were molded into the shape of contact lenses and inserted under the eye lids(8). The same results were obtained with circular aluminum foil electrodes of 6 cm in diameter placed on the dog's head. These monopolar electrodes and an indifferent electrode from the tongue of the animal, were connected with a rheoencephalograph.† The instrument measures impedance using a constant current of 0.002 amp at 30 kilocycles and approximately 0.7 volts are modulated by the pulse volume to a wide range of milliohms. Calibrations of 50 and 200 milliohms are available. Simultaneous tracings of the rheoencephalogram from both sides of the head, the blood flow and pressure in the outflow line of the pump were recorded on a Sanborn multichannel instrument. After adequate blood flow and oxygenation were established in the extracorporeal circulation, the cardiac output of the animal was stopped by externally induced ventricular fibrillation. Thus, the perfusion of the isolated dog's head

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