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Lack of Renal Effects of DOCA, ACTH, Spironolactone, and Angiotensin II in *Squalus acanthias*

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ABSTRACT Angiotensin II was infused intravenously in spiny dogfish sharks (*Squalus acanthias*). There were no significant effects on arterial blood pressure, glomerular filtration rate, urine flow, or Na excretion either in comparison with pre- and postinfusion values or in comparison with values measured in a control group of fish given elasmobranch saline intravenously. In other dogfish, glomerular filtration rate, urine flow, and Na and K excretory rates were measured for 3 days following implantation of desoxycorticosterone (DOCA), adrenocorticotropin (ACTH), or spironolactone; a control group was given no drug. There were no significant differences between these four groups of fish with respect to any of the measured parameters. These results suggest that the dogfish kidney is not a target organ for several substances known to affect renal function, either directly or indirectly, in other animals.

In all mammals studied, the renin-angiotensin-aldosterone system is of major importance for regulating salt and water balance. Aldosterone enhances Na reabsorption and K secretion in the distal nephron (Gross, '74). Angiotensin II increases blood pressure and thirst and stimulates the secretion of aldosterone and antidiuretic hormone (Blair-West et al., '74; Davis, '74; Dickinson and Ferrario, '74). In addition to the renal effects which are mediated by changes in hemodynamics or aldosterone secretion, angiotensin may have direct effects on renal tubular transport, inhibiting Na and water reabsorption when plasma levels are high and stimulating reabsorption at lower, physiological levels (Vander, '63; Munday et al., '72; Gross and Mohring, '73; Johnson and Malvin, '77).

Nonmammalian vertebrates (bony fishes, amphibians, reptiles, birds) have the renin-angiotensin system (Nishimura, '80) and secrete mineralocorticoids (Chester-Jones et al., '62; Holmes et al., '63; Denton, '65). Control of mineralocorticoid secretion is incompletely understood, but the renin-angiotensin system (Taylor and Davis, '71) as well as an ACTH-like hormone from the pituitary (Nandi and Bern, '60; Chester-Jones et al., '62; Denton, '65) might be involved. Elasmobranchs secrete steroids (Bern et al., '62; Idler and Truscott, '69) but lack the renin-angiotensin system (Nishimura, '80). However, injection of high doses of angiotensin II (20–40 μg bolus) does increase arterial blood pressure in the dogfish shark *Squalus acanthias* (Opdyke and Holcombe, '76).

These experiments were done to determine if 1) DOCA, a potent, mineralocorticoid 2) ACTH, presumably via stimulated endogenous corticoid secretion, 3) spironolactone, a mineralocorticoid inhibitor, or 4) angiotensin II at low doses have any renal effects in the dogfish shark.

MATERIALS AND METHODS

Female dogfish were captured by hook and line in the vicinity of Salisbury Cove, Maine. They were transported to the laboratory in running sea water and kept in live cars until used. Only animals in apparent good condition were used, usually within 2–3 days following capture.

Acute experiments

Approximately 24 hours before the experiment was begun, 1 ml/kg body weight of a 10% solution of inulin was injected IM at several site. On the experimental day, the

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fish was restrained in a tank filled with running sea water. A small ventral incision was made after infiltrating the area with lidocaine, and hepatic portal vein and celiac artery were catheterized with polyethylene tubing. No overt sign of pain was exhibited by any fish; they all lay quietly during the operative procedure. The arterial catheter was connected to a U-tube manometer filled with an elasmobranch saline solution (Lutz, '30) and arterial blood pressure was recorded every 5–10 minutes throughout the experiment. The venous catheter was used for administering solutions. Polyethylene tubing was also inserted into the urinary papilla and tied securely.

At zero time, an intravenous infusion was begun, consisting of saline at 0.11 ml/min. About 45 minutes later, the control clearance period was begun. Urine was collected for approximately 60 minutes in a graduated test tube, and arterial blood was sampled (about 0.4 ml) at the clearance midpoint. Following this, angiotensin II was added to the infusate; the rate of administration ranged from 18–36 ng/min/kg body weight. Thirty minutes later, the 60-minute experimental clearance period was begun. Finally, the infusion was changed back to saline alone, and 30 minutes later, a 60-minute recovery clearance period was begun. Fish were sacrificed and weighed after the third clearance period. A second group of dogfish was treated identically, but received only elasmobranch saline during the three clearance periods. These fish served as the control group.

Chronic experiments

Ten to fifteen hours before the first experimental day, female dogfish were pretreated in one of the following ways. 1) A 3–5cm abdominal incision was made, as described above, and a DOCA-impregnated wafer of silastic rubber was implanted in the peritoneal cavity. The DOCA content of the wafers ranged from 46–179 mg, and averaged 109 ± 10 mg. 2) Silastic rubber without DOCA was implanted.) The silastic implants were impregnated with ACTH (H.P. Acthar Gel; Armour Pharmaceutical, Phoenix, Arizona). ACTH content ranged from 138 to 370 Units and averaged 223 ± 20 Units. 4) The implants contained ACTH vehicle. The abdominal incisions in these dogfish were then tightly sutured. 5) An aqueous slurry, containing 25 mg of spironolactone (Aldactone; Searle and Co., Skokie, Illinois) was injected IP. Then the urinary papillae of all fish were catheterized with polyethylene tubing. The catheters were wound with adhesive tape at the other end, so that they could be fitted with rubber balloons for urine collection. Inulin was injected as described above. The next morning, the fish was restrained long enough to puncture the dorsal aorta and draw a blood sample (about 0.4 ml) and to attach a rubber balloon. Then the fish was released and allowed to swim freely in a large tank of running sea water. Four to six hours later, the fish was recaptured, a blood sample was taken, and the balloon was removed. Then one half the original dose of inulin was injected. This protocol was repeated for 2 more

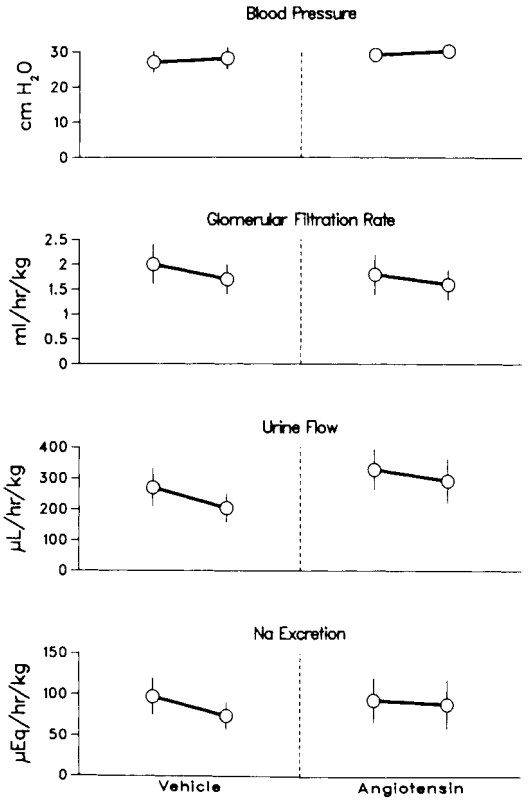

Fig. 1. Lack of effects of intravenous angiotensin II on mean arterial blood pressure, glomerular filtration rate, urine flow, and Na excretion in the dogfish. The experimental group (N = 11; right panel) received angiotensin during the second of three consecutive clearance periods, and elasmobranch saline during the first (control) and third (recovery). Values during the first and third periods were averaged, and are depicted as the first of the two connected points. The control (N = 5; left panel) group received only the vehicle (elasmobranch saline) during the three consecutive clearance periods. Values are mean ± SEM.

days. At the end of the third clearance, the fish was restrained and the dorsal aorta was punctured with a spinal needle. Polyethylene tubing was threaded into the artery, then connected to a U-tube manometer filled with saline. After recording blood pressure for a few minutes, the fish was killed and weighed.

Chemical analyses and calculations

Na and K were determined by flame photometry using Li as an internal standard. Inulin was determined by a colorimetric method (Harrison, '42). Plasma desoxycorticosterone (DOC) was measured by a radioimmunoassay using antiserum obtained from Dr. Guy Abraham (Torrence, CA). The plasma samples were extracted twice with ethyl ether. The aqueous layer was frozen and the solvent decanted, dried, and reconstituted in isooctane which was saturated with ethylene glycol. These samples were then applied to a celite column. The eluate was dried, reconstituted in ethanol, and applied to Whatman #1 paper coated with 25% propylene glycol in acetone, and run in toluene saturated with propylene glycol. The DOC area was identified by a radiochromatogram scanner and extracted with ethanol. These extracts were then assayed by radioimmunoassay.

RESULTS

Acute experiments

The injection of inulin several hours previously resulted in relatively constant plasma concentrations during the acute experiments. These experiments lasted 5 to 6 hours, and plasma inulin averaged 19 $\pm$ 4 and 16 $\pm$ 3 and mg% at the beginning and end, respectively.

The results of infusions of angiotensin II dissolved in saline and of saline alone are shown in Figure 1. Measurements made during the first (control) and third (recovery) clearance periods were averaged. It can be seen that angiotensin II, at the dosages used, had no significant effects on arterial blood pressure, glomerular filtration rate, urine flow, or Na excretion ($P > 0.05$, paired t test). Moreover, there were no significant differences in these parameters between these fish and those that received saline alone ($P > 0.05$, unpaired t test).

Chronic experiments

There were no significant differences with respect to glomerular filtration rate, urine flow rate, Na and K excretory rates, and urinary Na/K between dogfish implanted

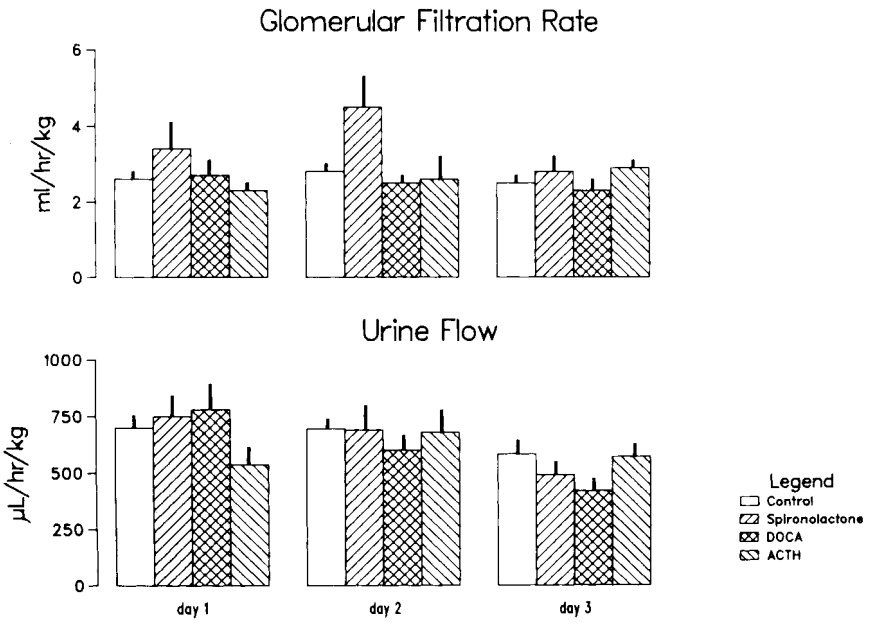

Fig. 2. Glomerular filtration rate and urine flow in dogfish injected with spironolactone in dogfish given DOCA- and ACTH-containing implants and in control dogfish, as indicated. The three groups of columns represent measurements on three consecutive days. Values are mean + SEM.

with silastic alone (as a control for the DOCA group) and those implanted with silastic containing the ACTH vehicle ($P > 0.05$, unpaired t test). Therefore, the data from these two groups were combined to form a single control group. It can be seen in Table 1 that averages of body weight were similar in the four resulting groups and that averages of arterial blood pressure, measured on the final day of the experiment, were virtually identical in the four groups.

TABLE 1. Body weight (BW) and mean arterial blood pressure (MAP) in dogfish

Group	BW (kg)	MAP (cm H_2O)
Control, N = 24	5.1 ± 0.2	28 ± 1
Spironolactone, N = 11	4.2 ± 0.3	28 ± 1
DOCA, N = 11	4.8 ± 0.3	27 ± 2
ACTH, N = 11	5.4 ± 0.2	28 ± 2

Results are mean ± SEM.

Glomerular filtration rates and urine flow rates of the four groups are shown in Figure 2. The glomerular filtration rate of the spironolactone group was higher than that of the other groups on the second day ($P < 0.05$), but otherwise there were no significant differences between the controls and the other groups.

Urinary Na and K excretory rates and urinary Na/K are shown in Figure 3. There were no significant differences in Na and K excretory rates between the four groups on any day. As an index of the renal effects of

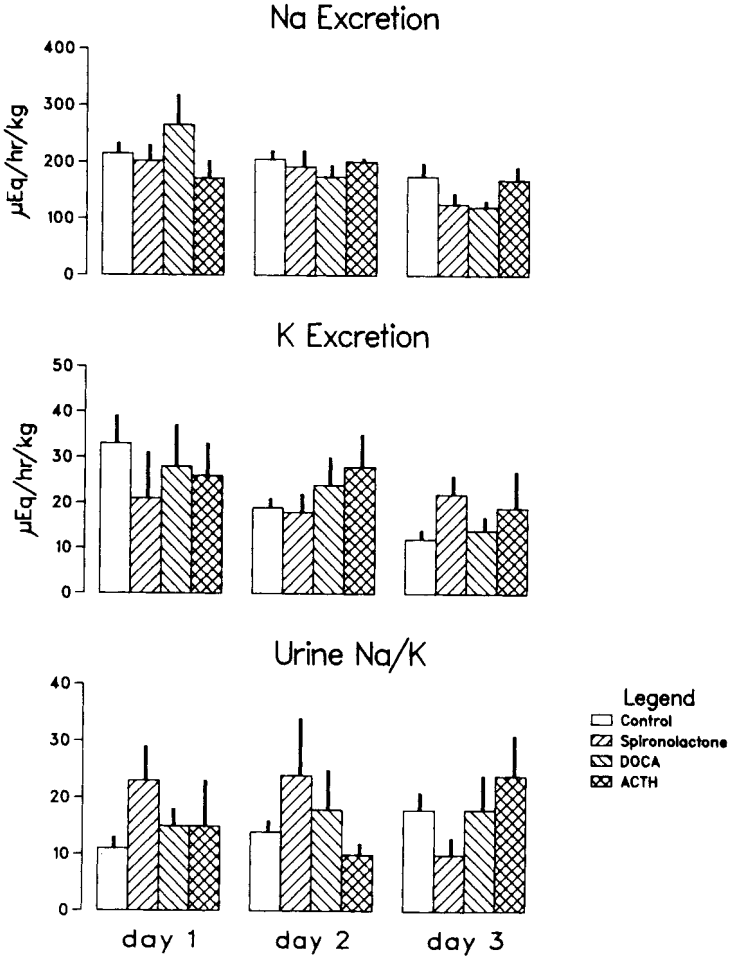

Fig. 3. Na and K excretion rates and urinary Na to K ratios in dogfish injected with spironolactone in dogfish given DOCA- and ACTH- containing implants and in control dogfish, as indicated. The three groups of columns represent measurements on three consecutive days. Values are mean + SEM.

steroids, urinary Na/K would be expected to be more sensitive than Na or K excretory rates, since the latter depend not only on accurate measurements of concentrations but also on accurate measurement of urine flow rate. As can be seen in Figure 3, spironolactone did not increase the ratio, or did either DOCA or ACTH decrease it, in comparison with control. Thus, spironolactone does not inhibit an endogenous renally acting steroid, DOCA has no demonstrable renal effects, and ACTH does not stimulate the secretion of a steroid which affects renal Na and K excretion as does aldosterone.

Plasma DOC was estimated in eight DOCA-implanted dogfish and in three controls. Averages were 383 ± 111 and 49 ± 10 ng/dl, respectively ($P < 0.05$).

DISCUSSION

Hickman and Trump ('69) have compiled, from several publications, the average values for renal function in *Squalus acanthias*. As measured with inulin, glomerular filtration rates range from 0.2 to 12 ml/hr/kg, and average about 3.5 ml/hr/kg. Typically, from 15 to 20% of the filtrate escapes reabsorption, such that urine flow averages can range from 500 to 1,000 μl/hr/kg. Na excretion ranges from 200 to 300 μEq/hr/kg. The averages of glomerular filtration rate, urine flow, and Na excretion that we calculated for the control group (2.6 ± 0.2 ml/hr/kg, 699 ± 56 μl/hr/kg, and 215 ± 18 μEq/hr/kg, respectively, on the first day) are thus in reasonable agreement with the previous results of others.

In mammals, mineralocorticoid secretion from the adrenal cortex is controlled by the renin-angiotensin system, plasma Na and K concentrations, and ACTH (Blair-West et al, '74; Davis, '74). Mineralocorticoids have a major action on the kidney, stimulating Na reabsorption and K secretion in the distal nephron (Gross, '74). Prolonged high plasma levels lead not only to Na retention and hypokalemia, but also to hypertension (Gross and Mohring, '73).

Less is known about steroids, control of steroid secretion, and the renal effects of steroids in elasmobranchs. The interrenal gland of dogfish is capable of synthesizing steroids (Bern et al., '62; Idler and Truscott, '69; Simpson and Wright, '70), and although the major steroid in plasma is reported to be 1α-hydroxycorticosterone (Idler and Truscott, '69), the interrenal gland may (Bern et al., '62) or may not (Simpson and Wright, '70) be capable of synthesizing aldosterone. Since the renin-angiotensin system is absent in dogfish (Nishimura, '80), steroid production and secretion must be controlled by another mechanism, possibly by an ACTH-like hormone. Dogfish adrenocorticotrophin and mammalian ACTH differ minimally; there are only two amino acid substitutions in the 1-19 region, and this region is thought to be all that is required for biological activity (Lowry et al., '74; Lowry and Scott, '75). Mammalian ACTH will stimulate steroidogenesis by interrenal tissue from bony fishes in vitro (Chester-Jones et al., '62; Nandi and Bern, '60). In the experiments of Bern et al. ('62), mammalian ACTH increased aldosterone production from incubated dogfish interrenal gland tissue, but only one trial was performed, and the capacity of dogfish interrenal glands to synthesize aldosterone has since been questioned (Simpson and Wright, '70).

With certain reservations, our results do not support a renal role for steroids in dogfish. DOCA implants raised endogenous levels of DOC approximately sevenfold, and this could not be shown to affect renal function in any way. However, it could be argued that endogenous levels were sufficiently high to exert a maximal effect. Although aldosterone does not appear to be the major endogenous steroid in dogfish plasma (Idler and Truscott, '69) and spironolactone is a competitive antagonist of aldosterone (Goodman and Gilman, '75), our results allow at least the conclusion that spironolactone does not antagonize the effects of any endogenous renally acting steroid. It is not clear why glomerular filtration rate was higher in the spironolactone-treated group than in the other groups on the second day; since this was not the case on either the first or the third days, and since spironolactone is not known to produce renal hemodynamic effects, it is difficult to attribute much physiological significance to the finding. Finally, mammalian ACTH did not stimulate the production and secretion of any steroid which in turn had detectable renal effects.

Angiotensin II has vascular and renal effects in bony fishes (Churchill et al., '79), amphibians (Coviello, '69), birds (Langford and Fallis, '66), and mammals (Vander, '63; Munday et al., '72; Gross and Mohring, '73; Johnson and Malvin, '77). As noted above, the renin-angiotensin system is absent in elasmobranchs (Nishimura, '80). However, 20–40-μg bolus doses of angiotensin II increase arterial blood pressure in dogfish, and

it has been suggested that this hypertensive effect is mediated by angiotensin-induced release of catecholamines, rather than by a direct vascular effect of angiotensin II (Opdyke and Holcombe, '76). Collectively, these results suggest that dogfish chromaffin tissue, but not dogfish vascular smooth muscle, possesses angiotensin II receptors. Since angiotensin II had no apparent renal effects, our results suggest that dogfish renal tubular cells also lack angiotensin II receptors.

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