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Renal Effects of DOCA (Desoxycorticosterone Acetate) in *Squalus acanthias*

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The effects of DOCA in mammals are well known. There is initially decreased sodium excretion and increased potassium excretion followed by expansion of extracellular fluid volume and increased blood pressure. Later sodium excretion rate increases believed due, at least in part, to increased glomerular filtration rate. Although the presence of mineralocorticoids in elasmobranchs was suggested many years ago by Denton (Physiol. Rev. 45: 245, 1965), to our knowledge the effects of DOCA administration in dogfish sharks have not been reported previously.

The dogfish used in this study were captured by trawl and then kept in cold aerated sea water. A total of 21 females, (4.69 ± 0.14 kg = mean body weight ± SEM) were used. Polyethylene tubing (PE 90) was inserted into the urogenital papilla and tied securely. Urine was collected into a balloon attached to these catheters. Blood pressure measurements (mercury manometer) and blood samples were obtained using an indwelling polyethylene catheter in the dorsal tail artery.

A 10 gram % solution of inulin was injected i.m. at several sites (total dose 1 ml/kg) at least 10 hours prior to urine and blood sampling. Duration of the urine collection periods averaged 6 hours for a total of 122 clearance periods. Blood samples were taken at the beginning and at the end of each period.

After 2-4 "control periods," a small (3-5 cm long) abdominal incision was made. A pre-weighted thin wafer of DOCA-implanted silastic rubber was implanted in 13 fish; the remaining 8 served as sham-implanted controls. The incisions were tightly sutured, and several clearance periods followed over the next 1-4 days. At sacrifice, the silastic-DOCA implants were removed, dried for several days, and reweighed.

Sodium and potassium in urine and plasma were determined by flame photometry using an internal Li^+ standard. Osmolality was determined by freezing-point depression (Advanced Osmometer). Inulin in urine and TCA plasma filtrates were determined by the diphenylamine method of Harrison (Proc. Soc. Exp. Biol. Med. 49: 111, 1942). For calculation of GFR, plasma inulin concentrations were determined by extrapolation to the clearance period midpoint. A photometric method was used for urea determinations (Am. J. Physiol. 222: 489, 1972). Plasma DOCA in 8 experimental and in 3 sham-operated dogfish was determined by radioimmunoassay; the averages were 583 ± 111 and 49 ± 10 ng %, respectively. As appropriate, the results were factored by body weight.

The intent was to use each animal as its own control, before and after the implant or sham-implant. However, by paired t-test statistics, we showed that the parameters measured in the sham-implant group had no tendency to change over time, nor was this group different in any respect from the other group prior to DOCA-implantation (unpaired t-test). Therefore, averages for each fish were calculated: control and/or sham-implant and DOCA-implant. These results are presented in Tables 1 and 2.

It was most surprising to find neither a sodium retaining nor a potassium loosing effect of DOCA. In fact, although these were not statistically significant, tendencies were in the opposite direction ($p > 0.1$). Although the increase in plasma osmolality achieved significance ($p < 0.02$), the trends for increases in measured plasma solutes were not statistically significant ($p > 0.2$). Urine flow rate increased ($p < 0.04$) and urine osmolality decreased. The increase in plasma osmolality cannot be explained by renal effects of DOCA since the product, $U_{osm} \cdot V$, actually increased in the DOCA-implant group.

In contrast to its long-term effects in mammals (2-4 days), DOCA did not produce hypertension, nor was there a tendency for GFR to increase (one of the factors presumed to mediate the "escape phenomenon" in certain mammals).

Pending confirmation, these results suggest that (1) if DOCA and aldosterone have similar or identical mechanisms and sites of action, and (2) if aldosterone plays some role in

Table 1

Effects of DOCA on Blood Pressure and Plasma Composition

	Blood Pressure mm Hg	Osmolality mOs/Kg H_2O	Na mEq/L	K mEq/L	Urea mM/L
Controls	28.7 ± 5.2	962 ± 37	256 ± 13.6	4.4 ± 0.8	347 ± 27
DOCA Implants	28.4 ± 4.4	991 ± 11	258 ± 11.0	4.5 ± 0.5	356 ± 24

MEANS ± SEM

Table 2

Renal Effects of DOCA

	GFR ml/Kg/Hr	Urine Flow ml/Kg/Hr	U_{osm} mOs/Kg H_2O	$U_{Na}V$ μEq/Kg/Hr	U_KV μEq/Kg/Hr	$U_{urea}V$ μM/Kg/Hr
Controls	1.9 ± 0.5	0.34 ± 0.08	760 ± 151	91 ± 32	25 ± 22	19 ± 13
DOCA	1.7 ± 0.8	0.46 ± 0.18	703 ± 112	120 ± 57	15 ± 8	23 ± 12

MEANS ± SEM

sodium and potassium balance in dogfish, then both role and major site of action contrast with those in mammals.

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The Length Tension Relation in the Single Cell Layered Myocardium of *Boltenia ovifera*

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The heart of the tunicate ***Boltenia ovifera*** (the sea potato) consists of a straight tube which propels blood through a primitive circulatory system by means of a peristaltic contraction. In the MDIBL Bulletin of 1972, 1973. and 1974, and in SCIENCE (186: 750, 1974), we have reported on some of the contractile and electrical properties of this single cell layered myocardium. In the present report, we have attempted to investigate the length-tension characteristics of this myocardium under conditions where length of the preparation was clamped to various values.

The sea potato heart was removed from the animal and was cannulated at one end. The pericardium was dissected away and an incision was made along the longitundinal raphe of the heart. This procedure produces a single cell layered sheet of muscular tissue. The sheet was placed over the opening of a lucite chamber separating the solution on the luminal side of the preparation electrically and mechanically from the solution of the extraluminal side. A silicone paste was applied around the edges of the opening to electrically seal the injured areas of the myocardium. The longitudinal ends of the myocardial preparation were snared down with nylon strings into two grooves running close to the opening covered with the tissue. The short ends of the preparation were then pressed down with two silastic wedges mounted on two micro-manipulators. With the preparation in place, Evans blue dye was injected into the bottom compartment of the chamber to check for leaks around the edges and the mechanical continuity of the preparation. The lower chamber pressure was slightly increased as to produce a gentle "bulge" in the myocardial preparation (see Figure 1 B). The height of the bulge could be observed on a calibrated grid in the dissection microscope. A light beam passing through the upper chamber (Figure 1 A) and focused in a vertical slit on the muscle was used to give an automatic measurement of the bulge height. The blue dye injected into the lower chamber was essential to increase the light blocking capabilities of the bulge as the tissue is highly transparent. The light source was an array of 9 light emitting diodes. The light source was chopped electrically at 40 KHz frequency. A phase lock amplifier was used to increase the signal to noise ratio of the measured transmitted light.

Increasing the height of the bulge decreases transmitted light. The pressure difference across the tissue was measured with a Statham pressure strain guage connected to the closed lower chamber (the surface of the fluid in the open upper chamber was held at a constant level). Eliciting a contraction by transepithelial electrical stimulation the height of the bulge decreases, thus increasing the transmitted light. The time course of transmission of light is directly related to the time course of the shortening of the myocardial preparation. The lower trace and the upper trace of Figure 2 A show the time course of pressure and transmitted light. Consistent with our previous observation we found that only depolarization of the luminal membrane produces contraction. The transepithelial resistance range between 75-200 Ω cm^2. Unlike the preparation used in the previous years, the sheet preparation did not show the graded contractile response with increasing stimulus strength.

In order to maintain a constant length during the time course of contraction, servo-control was applied to keep the bulge height constant. The light signal measured with the photodiode was compared with an adjustable reference and the difference was amplified and fed to a servo-controlled motor (Gould Brush, Mark 220) converting the electrical signal to an angular position. A crank shaft attached to the axis of the motor was hinged to a rod in rigid connection with a piston which displaced sufficient fluid to clamp the height of the bulge. The DC loop gain of the system was approximately 10. Effective clamp control of the height of the bulge could often be obtained within 25 msec. Figure 2 A shows the time course of contraction and the light signal with the loop open (top and bottom traces), and with the loop closed (middle two traces). Note that the light signal hardly changes when the loop is closed and that the pressure signal is strongly enhanced.

Figure 2 B shows the isometric pressure records from the bulge-clamped preparation where the bulge height is changed stepwise between individual recordings. As the height of the bulge and thereby the length of the preparation is decreased, the maximum twitch pressure decreases. A resting pressure is only observed for the first three records where the muscle was stretched considerably. Laplaces law was applied to derive the wall tension, T, from the pressure, P, and the radius of bulge curvature, r. The bulge was assumed to approximate a segment of a circular cylinder. Very little curvature is observed in the central region when the bulge is observed from the side (see Figure 1 B). The radius of the cylinder was estimated according to the schematic shown in Figure 1 C, also showing the relation between the bulge height and the length of the preparation, 1.

In Figure 1 B, the twitch pressure obtained with maximal bulge height (h = 1.2 mm) is P = 1.5 cm H_2O = 1.5 g/cm^2. The radius of the bulge is r = $(1.2^2 + 1.125^2) / (2 \cdot 1.2)$mm =