STUDIES OF THE LOCI OF STIMULATION OF ALDOSTERONE BIOSYNTHESIS DURING SODIUM DEPLETION

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It is well established that synthesis of aldosterone can be stimulated by ACTH and by angiotensin; in addition, it can be stimulated by increases of potassium ion concentration and decreases of sodium ion concentration in the fluid immediately surrounding the adrenal cortex. It is not clear whether these factors alone can explain the physiological control of aldosterone synthesis, readily increased by such procedures as depletion of body sodium.

In the present studies, the effects of sodium depletion on the biosynthesis of adrenal steroid hormones in the hypophysectomized dog were explored by in vivo and in vitro techniques in an attempt to identify the loci at which sodium depletion stimulates aldosterone production. The in vivo studies indicate that sodium depletion stimulates the secretion of aldosterone, corticosterone, and cortisol by the hypophysectomized dog. This suggests that the steroid biosynthetic pathway is stimulated by salt depletion at an early stage; this effect could depend upon stimulation by the renin-angiotensin system (Davis, J.O. et al, 1961; Binnion, et al, 1965; Slater, et al, 1963; Mulrow, et al, 1962). When adrenal slices were prepared from the same dogs, sodium depletion again appeared as a strong stimulus to production of aldosterone, but adrenal slices from sodium-depleted dogs produced significantly less corticosterone than slices from sodium-replete animals. This finding, together with estimates of in vitro corticosterone "pool" size with radioactive

precursors, suggests that the conversion of corticosterone to aldosterone is stimulated by sodium depletion. This effect does not depend upon changes in potassium or sodium ion concentration.

MATERIALS AND METHODS

Mongrel dogs were hypophysectomized by the buccal approach and either fed a high-sodium diet (220 mEq a day) or depleted of sodium by injection of 2 ml of mercuhydrin intramuscularly and then fed a low-sodium diet (8 mEq a day). Two days later the lumboadrenal vein was cannulated under nembutal anesthesia (26 mg/Kilo) and secretion rates of aldosterone, corticosterone and cortisol were determined. The adrenals were then removed and slices weighing about 100 mgm were prepared from the outer surface with a Stadie-Riggs microtome. Each slice was preincubated for one hour in 5 ml of Krebs-Ringer bicarbonate buffer containing 200 mg per cent glucose in a metabolic incubator equilibrated with oxygen containing 5 per cent CO_2. The medium was then discarded and replaced with fresh buffer containing the desired precursor. Subsequent incubation was carried out for two hours.

Aldosterone, corticosterone, and cortisol were determined by the method of Kliman and Peterson (1960), with the following modifications. After acetylation, a preliminary thin-layer chromatography was done on silica gel with chloroform ethanol (96:4) as the developing solution. The steroids were located under ultraviolet light with the aid of rhodamine and eluted with acetone ethanol (1:1). A fifth chromatographic separation was required for the acetates of corticosterone and cortisol to obtain a low blank and stable $H^3:C^{14}$ ratio. This was done on the benzene and dioxane systems of Kliman and Peterson before and after chromate oxidation. Ring-labeled aldosterone, corticosterone and cortisol were added before extraction and used to measure recovery. Tritium and carbon ^{14} were assayed in a Packard Tri-Carb liquid scintillation counter.

RESULTS AND DISCUSSION

TABLE I

	Aldosterone mμg/min.	Corticosterone mμg/min.	Cortisol mμg/min.
Sodium Replete n=7	16±3*	13±2	22±9
Sodium Depleted n=7	138±39	184±66	103±29

*1 standard error of mean.

Effect of sodium depletion on secretion of steroids in the hypophysectomized dog.

Table I shows the adrenal vein secretion rates in control and sodium-depleted dogs. Sodium depletion produced a ten-fold increase in the secretion of aldosterone and corticosterone and two to three-fold increase in cortisol production. These studies suggest that the early part of the biosynthetic pathway of aldosterone is stimulated by sodium depletion and are in agreement with previous studies in the dog (Davis, J.O. 1961; Binnion et al, 1965) and in part with studies in man (Bledsoe et al, 1965). This pattern of steroid secretion is compatible with stimulation of aldosterone secretion by the renin-angiotensin system (Slater, et al, 1965) in sodium depletion, and is indirectly supported by the finding that plasma renin activity is increased by sodium depletion (Brown, et al, 1963; Davis, J.O., et al, 1961; and Binnion, et al 1965)

Table II shows the results of *in vitro* incubation of adrenal slices from the same dogs. When no precursor was used, aldosterone production increased and corticosterone production *decreased*, both by a factor of two, while cortisol output did not change. If aldosterone biosynthesis proceeds through the sequence: Cholesterol → Δ-5 pregnenolone → progesterone → desoxycorticosterone → corticosterone → aldosterone, the increase in aldosterone secretion with the decrease in corticosterone secretion suggests a stimulation of the conversion of corticosterone to aldosterone. A similar pattern of steroid output has been observed recently with sodium depletion in the rat (Cade and Perenich, 1965), in which aldosterone secretion is stimulated minimally or not

at all by the renin-angiotensin system (Eilers and Peterson, 1964; Kaplan and Bartter, 1962; Cade and Perenich, 1965; Merieb and Mulrow, 1965). It has not been reported for the dog, in which aldosterone production is stimulated by angiotensin.

TABLE II

	Precursor added	Aldosterone µg/g 1 hr.	Corticosterone µg/g 1 hr.	Cortisol µg/g 1 hr.
Sodium replete n=12	None	3.7±0.5	3.2±0.26	1.9±0.4
Sodium replete n=2	None +ACTH-lu.	4.8	7.4	19
Sodium depleted n=12	None	7.3±1.1	1.6±0.60	1.6±0.2
Sodium replete n=11	Progesterone	5.0±0.46	12.6±1.1	17.2±1.0
Sodium depleted n=11	Progesterone	10.3±1.0	13.4±0.6	18.1±1.7
Sodium replete n=8	Corticosterone	7.2±0.8	-	3.3±0.5
Sodium depleted n=8	Corticosterone	12.8±2.4	-	4.9±0.8

Effect of sodium depletion on the production of aldosterone in the presence and absence of non-radioactive precursors by dog adrenal slices. Added progesterone and corticosterone: 25 µg in 25 µl of alcohol.

The existence of a second, "distal" locus for stimulation of aldosterone secretion is supported by the experiments listed in table 2. When progesterone or corticosterone, aldosterone precursors "distal" to the locus of action of angiotensin, were added, aldosterone production was stimulated; slices from sodium-depleted dogs showed significantly more biosynthesis of aldosterone than slices from sodium-replete dogs. Cortisol and corticosterone production were stimulated to a comparable extent by progesterone in both groups, and this response was the same as that observed with stimulation by ACTH. These effects of sodium depletion are in contrast with the effects of angiotensin and ACTH on bovine adrenal slices: both hormones can stimulate biosynthesis of steroids in the presence of precursors "prior to" △-5 pregnenolone but have no effect with precursors "distal" thereto (Kaplan and Bartter, 1962).

TABLE III

H^3 in aldosterone DPM/100 mgm/2 hr.

Sodium Replete n=12	29,750±2,400	Sodium Depleted n=12	51,075±5,000

In vitro incorporation of 1,2 H^3 corticosterone.

Slices were incubated as in "Methods", except that 1.35 μc of 1,2 H^3 corticosterone were added in 50 μl of ethanol. 4-C^{14} aldosterone was at end of incubation to correct for recovery. The medium was extracted with methylene chloride, acetylated with non-radioactive acetic anhydride and the aldosterone purified to constant specific activity as in "Methods".

Table III showes the results of in vitro incorporation of tritium-labeled corticosterone of high specific activity into aldosterone by slices from sodium-replete and sodium-depleted dogs. Sodium depletion produced a two-fold stimulation of incorporation of exogenous, labeled corticosterone into aldosterone. All of these results thus suggest that the conversion of corticosterone to aldosterone is increased by salt depletion. The mechanism by which this biosynthetic step is controlled is not known.

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