Inhibitory Effect of Ethanol upon Aldosterone Synthesis by Beef Adrenal Tissue[1] (35895)

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Ethanol has been found to stimulate adrenocortical steroid secretion in animals (1) and man (2). Since the effect occurs only in the presence of an intact pituitary, the action of ethanol *in vivo* is thought to involve the release of ACTH, perhaps through a hypothalamic pathway.

In the course of our studies upon adrenal steroidogenesis *in vitro*, we used small amounts of ethanol to solubilize various stimulatory agents. An apparent inhibition of the expected responses was observed. Since there are no known published data on the *in vitro* effects of ethanol upon adrenal steroidogenesis and no known data on either *in vivo* or *in vitro* effects of ethanol upon aldosterone synthesis, additional studies were performed to characterize further the effects of ethanol upon adrenal steroid synthesis.

Material and Methods. The experiments utilized an *in vitro* technique that has provided data closely paralleling the results of *in vivo* studies in animals and man as to the effects of ACTH, angiotensin, and potassium (3).

The two outer slices of the cortex of 6 to 8 beef adrenal glands were taken with a microtome. The slices were combined and minced to obtain uniformity. At least four vessels containing 500 mg of tissue were incubated and analyzed separately for each portion of each experiment including a control set. In all experiments, the tissues were preincubated at 37° for 1 hr in 5 ml of Krebs–Ringer bicarbonate medium containing glucose at a concentration of 200 mg/100 ml under 95% O_2–5% CO_2. The preincubation medium was then replaced with 5 ml of fresh medium and appropriate agents were added. After a 2-hr incubation, the media were decanted and 1 ml was analyzed for aldosterone, corticosterone, and cortisol by the double isotope derivative assay of Kliman and Peterson (4). The assay technique was not changed except for the addition of known amounts of the appropriate ^{14}C-ring labeled steroids to each sample as internal standards at the onset of the assay instead of after acetylation and the use of two chromatographic separations both before and after oxidation of ^{3}H-labeled acetates of the different steroids. All values were expressed as micrograms of steroid per gram of beef adrenal tissue per 2-hr incubation. Statistical evaluation was by the *t* test.

Ethanol was redistilled absolute alcohol, USP grade. Angiotensin was synthetic asparaginyl-1, valine-5 angiotensin II (Ciba). ACTH was procine aqueous ACTH (Upjohn), known to be biologically effective. The concentration of electrolytes in the incubation media was determined by flame photometry. The Krebs–Ringer bicarbonated medium had a concentration of sodium of 142 mEq/liter, potassium of 5.5 mEq/liter, and an osmolality of 274 Osm/liter. The low sodium medium had a concentration of sodium of 101 mEq/liter, potassium of 5.4 mEq/liter, and an osmolality of 272 Osm/liter, the osmolality having been normalized by addition of 25% mannitol.

The effects observed could not be attributed to a selective effect of the ethanol upon extraction of the steroids from the media. The extraction of all 3 steroids from 1 ml of media was virtually complete with the 10 ml of dichloromethane used in the procedure.

Results. The presence of ethanol in a con-

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TABLE I. Effects of Various Concentrations of Ethanol in Incubation Media upon Steroid Synthesis in Beef Adrenal Tissue.

	Aldosterone (μg/g)	Corticosterone (μg/g)	Cortisol (μg/g)
Control	2.02 ± 0.33[a]	6.29 ± 0.38	3.63 ± 0.80
Ethanol, 1%	1.48 ± 0.11[b]	6.00 ± 0.50	4.20 ± 0.44
2%	1.05 ± 0.20[c]	5.92 ± 0.37	3.88 ± 0.51

[a] Each value is mean ± SD of 4 separate vessels.
[b] Compared to control: $p < .01$; [c] $p < .001$.

centration of 0.1% had no effect upon beef adrenal steroid synthesis; whereas, as shown in Table I, 1 and 2% concentrations significantly decreased the levels of aldosterone but did not affect the synthesis of corticosterone or cortisol. Similar results were obtained in two other experiments.

These results suggested that the effect of ethanol may be upon the conversion of corticosterone to aldosterone. To delineate further the possible site wherein ethanol inhibits the synthesis of aldosterone, its effect upon three stimuli of aldosterone synthesis was examined. Angiotensin II and ACTH stimulate adrenal steroidogenesis early in the biosynthetic pathway, at the level of cholesterol (5); whereas a decreased sodium concentration acts later, to increase the conversion of corticosterone to aldosterone (6).

Ethanol, 2%, significantly decreased the synthesis of aldosterone by itself and in the presence of angiotensin II and a low sodium concentration (Expt. I, Table II and Fig. 1). Similar inhibition of the stimulation of aldosterone synthesis produced by ACTH was noted with 1% ethanol in Expt. II, Table II. The values of aldosterone synthesis in the presence of ethanol are all significantly less than those without ethanol ($p<.05$) with angiotensin, low sodium, and ACTH.

As shown in the last column of Table II, cortisol synthesis was unaffected by ethanol, alone or in combination with the various stimulatory agents. However, the effects upon corticosterone synthesis, shown in column 3, are in keeping with a site of action of ethanol somewhere between the conversion of corticosterone to aldosterone. Ethanol had no effect upon corticosterone synthesis by itself or with either angiotensin II or ACTH, which act early in the biosynthetic pathway. On the other hand, ethanol significantly ($p<.05$) blunted the decrease in corticosterone synthesis produced by a low sodium concentration in the tissue medium and returned the rate of aldosterone and corticosterone synthesis to

TABLE II. Effects of Angiotensin, ACTH, and Sodium Depletion Alone and with Ethanol upon Steroid Synthesis in Beef Adrenal Tissue.

	Agent	Aldosterone (μg/g)	Corticosterone (μg/g)	Cortisol (μg/g)
I.	Control	1.24 ± 0.12[a]	9.11 ± 0.98	1.77 ± 0.21
	Ethanol, 2%	0.81 ± 0.15[b]	8.54 ± 0.84	1.69 ± 0.19
	Angiotensin, 20 μg/g	1.83 ± 0.11[c]	13.62 ± 1.60[b]	2.18 ± 0.24[d]
	+ ethanol, 2%	1.49 ± 0.12	11.91 ± 1.00	2.00 ± 0.20
	Low sodium	1.59 ± 0.13[d]	7.11 ± 0.47[b]	1.89 ± 0.21
	+ ethanol, 2%	1.19 ± 0.13	9.05 ± 0.85	1.80 ± 0.24
II.	Control	1.06 ± 0.05	9.14 ± 0.55	1.19 ± 0.10
	ACTH, 2 units/g	1.24 ± 0.08[d]	11.22 ± 0.91[d]	1.87 ± 0.20[c]
	+ ethanol, 1%	0.81 ± 0.19	11.10 ± 0.84[d]	1.70 ± 0.10[d]

[a] Each value is the mean ± SD of 4 separate vessels.
[b] Compared to control: $p < .01$; [c] $p < .001$; [d] $p < .05$.

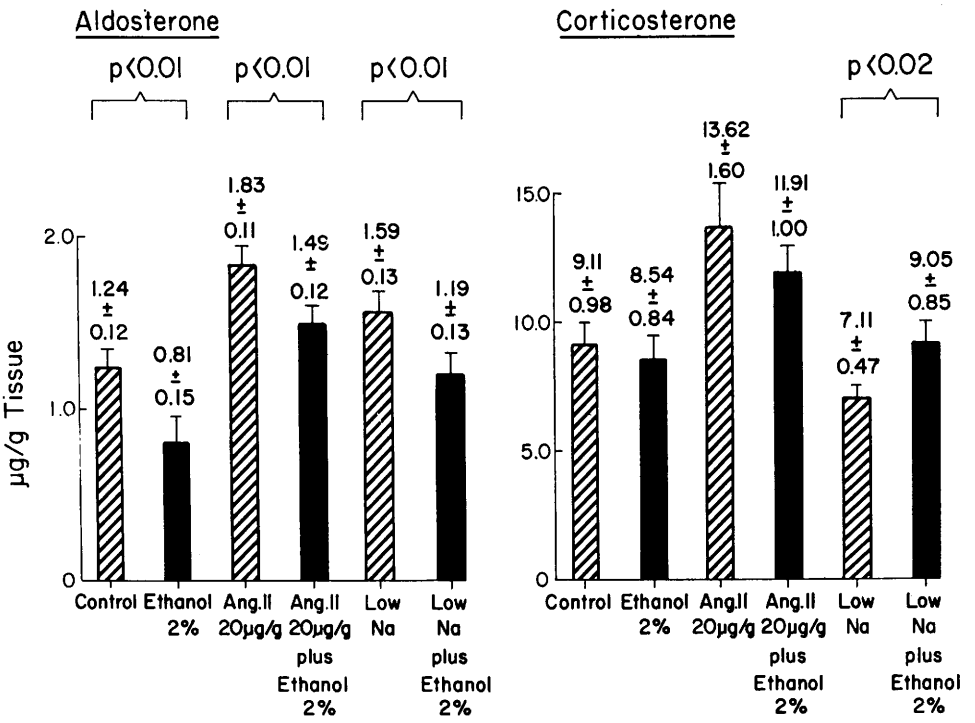

FIG. 1. The effect of ethanol alone and in combination with angiotensin II or a low sodium concentration upon the synthesis of aldosterone and corticosterone in beef adrenal tissue *in vitro*. The values are the mean ± SD. The *p* values compare each set of 4 vessels with and without 2% ethanol.

the control level. Thus ethanol appears to interfere with the conversion of corticosterone to aldosterone.

Discussion. These *in vitro* studies demonstrate an inhibition of aldosterone synthesis, presumably at the site of conversion of corticosterone to aldosterone. Obviously caution is advised in applying these *in vitro* results to *in vivo* physiologic mechanisms. The concentration of ethanol used was 1%, a level higher than that reached with severe intoxication in man. However, larger amounts of various agents are needed to exert effects in the artificial environment of such tissue experiments (5).

Nonetheless, these experiments provide a way of examining specific actions of various agents directly upon the adrenal without the modulating effects of the hypothalamic–pituitary axis and other systems. The modest direct inhibition of aldosterone synthesis observed with 1 or 2% ethanol is presumably not an important *in vivo* physiologic mechanism since a decrease in sodium excretion is observed when alcohol is given acutely to man (7). However the adrenals were not thought to play a role in the decreased sodium excretion since the effect was observed in a patient with adrenal insufficiency (8).

Even if ethanol exerts no important *in vivo* effect upon adrenal steroidogenesis, the *in vitro* effects demonstrated in this study suggest caution in its use as a carrier or solubilizing agent in studies upon hormonal synthesis.

Summary. Ethanol, 1 and 2%, inhibited the synthesis of aldosterone in beef adrenal tissue *in vitro*. The inhibition appears to occur between the conversion of corticosterone to aldosterone.

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