Effects of Estrogens on Pressor Responses to Angiotensin and Renin[1] (34484)

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There is increasing evidence that estrogens affect the renin–angiotensin–aldosterone system. They cause marked and consistent increases in plasma angiotensinogen concentration (1, 2), transient or sustained increases in plasma renin activity and in aldosterone secretion (2, 3), and usually a decrease in plasma renin concentration (4). *In vitro* studies suggest that the high plasma renin activity associated with the low plasma renin concentration is due to an increased ability of the plasma to generate angiotensin (2). If such is the case, estrogen treatment should result in an enhancement of pressor responses to administration of exogenous renin, but not of angiotensin. This we demonstrate in the present paper.

Methods. Female Sprague-Dawley rats weighing 180–240 g were used. They were fed Purina Fox Chow and given tap water as drink.

Experiment 1. Rats were divided into two groups of 18 animals each and ovariectomized. Three days later animals of the first group received a daily injection of 0.1 mg of diethylstilbestrol in oil during 4 days, while those of the second group received oil injections. On Day 5 six rats from each group were tested for pressor responsiveness to angiotensin and renin after amobarbital anesthesia. The same procedure was applied to another six rats except that they also received, prior to testing, one injection of 20 mg/kg of pentolinium tartrate. The remaining six animals were anesthetized with ether, and blood was withdrawn from the aorta in the presence of EDTA for determination of plasma angiotensinogen.

Experiment 2. Rats were divided into 2 groups of 12 animals each. Animals of the first group received one daily injection of 0.1 mg of diethylstilbestrol during 4 days at which time they, as well as the untreated animals of the second group, were bilaterally nephrectomized. The next day 6 rats from each group were tested for pressor responsiveness, without sensitization by pentolinium, and the other 6 rats were bled for determination of plasma angiotensinogen.

Measurement of pressor responsiveness. After anesthesia with sodium amobarbital (9 mg/100 g), the trachea was intubated, and the carotid artery was connected to a P23-db Statham Transducer for pressure recording on a Sanborn recorder. After stabilization of pressure, two doses of 5.5 ng of angiotensin amide (Hypertensin, CIBA) were injected into the femoral vein followed by 0.03 or 0.015 Goldblatt units of rat renin (5). Pressor responses are expressed in millimeters of mercury (mm Hg).

Measurement of angiotensinogen. Plasma (0.05 or 0.1 ml) was incubated with 1.25 Goldblatt units of rat renin under conditions described (5) and the angiotensin formed bioassayed in pentolinium-treated rats. Results are expressed in nanograms (ng) of angiotensin II per milliliter. All data presented are mean ± SE.

Results. In Expt. 1, pressor responses to angiotensin were quite reproducible within each group (Table I); they were not significantly affected by estrogen treatment ($p > .05$). However, pressor responses to 0.03 units of renin were regularly and significantly increased ($p < .005$). The same results were obtained in rats given one injection of pentolinium prior to testing, where again, only differences between pressor responses to renin

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TABLE I. Pressor Responses to Angiotensin and Renin in Estrogen-Treated Rats.

Procedure and treatment	Pressor responses (mm Hg) to: Angiotensin (5.5 ng) 1st Response	Angiotensin (5.5 ng) 2nd Response	Renin[a]
—	17.7 ± 1.28	19 ± 2.0	19.5 ± 2.06
Stilbestrol	15.7 ± 1.02	16.3 ± 1.3	31.0 ± 1.36
—[b]	28.0 ± 3.4	—	29.2 ± 3.08
Stilbestrol[b]	21.0 ± 1.2	—	42.0 ± 1.3
Nephrectomy	17.5 ± 1.19	16.8 ± 1.1	21.7 ± 1.93
Nephrectomy and stilbestrol	16.5 ± 1.2	16.5 ± 1.08	28.6 ± 2.3

[a] Renin (0.03 units per dose) in the first four groups and 0.015 units in the last two groups.
[b] Pretreatment with pentolinium.

in normal and estrogen-treated rats were statistically significant ($p<.005$). As expected, pentolinium made the animals more sensitive to both angiotensin and renin. Plasma angiotensinogen concentration was increased about three times by estrogen treatment (Table II).

Results from Expt. 2 (Table I) show that nephrectomy alone increased pressor responses to renin but not to angiotensin. Responses to 0.015 units of renin were about the same as those to 0.03 units in non-nephrectomized animals. Estrogen treatment prior to nephrectomy caused a further increase in responsiveness to renin, which was significantly different from control values in nephrectomized animals ($p < .05$) but to a lesser degree than in Expt. 1. Plasma angiotensinogen was increased about 5 times by nephrectomy and 14 times by nephrectomy plus estrogen treatment (Table II).

Discussion. Our results on pressor responses to angiotensin and renin after estrogen treatment are in agreement with the preliminary data reported for rats by Douglas *et al.* (6) after administration of estrogens, as contained in the contraceptive agent, Enovid. Lack of effect of estrogens on the pressor response to angiotensin had already been noted (7). The enhancement of responses to renin without concurrent increases in responses to angiotensin indicates that estrogens do not act by sensitizing the vascular system, but, rather, suggests an increase in angiotensin formation.

TABLE II. Effects of Estrogens on Plasma Angiotensinogen.

Procedure and treatment	Plasma angiotensinogen (ng/ml)
—	378 ± 36
Stilbestrol	1178 ± 135
Nephrectomy	1737 ± 106
Nephrectomy and stilbestrol	5250 ± 713

An increase in angiotensin formation can result from the removal of an inhibitor or release of an activator of the renin–angiotensin reaction. We have, however, no information on the effect of estrogens on such cofactors. The possibility that a different but more reactive substrate is released by estrogens cannot be entirely discarded, although we have no proof of its existence. The most likely possibility is that amounts of angiotensin formed are related to the consistent and remarkable increase in substrate elicited by estrogens, as shown (1, 2, 4, 8) and confirmed here. The general belief that amounts of substrate normally present are sufficient to permit the enzymatic reaction to proceed at maximum velocity seems to be refuted by the observation that the rate of angiotensin formation increases with increments in substrate concentration (2, 8). However, one may argue that conclusions from *in vitro* studies do not necessarily apply *in vivo*, where renin and substrate are continually secreted and angiotensin destroyed. Nevertheless, it has been suggested that sub-

strate concentration may be a controlling factor of renin secretion: an increase in substrate would release more angiotensin, which, in turn, would inhibit renin secretion so that the final effect would be a low renin concentration and a normal or elevated plasma renin activity (2, 4).

Differences in response to renin in estrogen-treated nephrectomized rats and in nephrectomized rats were not so great as expected on the basis of differences in substrate concentration. It may be that substrate is a less limiting factor on enzyme velocity when its concentration reaches a certain level. Thus, Gould *et al.* (9) found that, when human renin reacts on hog substrate, the substrate is rate-limiting in concentration up to 840 ng/ml. It is not known whether a similar situation would obtain when the reaction takes place between rat renin and rat substrate.

Summary. Administration of diethylstilbestrol to normal or nephrectomized rats causes an increase in angiotensinogen concentration in plasma associated with an increase in the pressor response to renin but no change in the pressor response to angiotensin.

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