EFFECT OF ESTRADIOL AND OTHER ENDOCRINE FACTORS ON THE PLASMA ANGIOTENSINOGEN LEVEL IN RATS

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UDC 615.256.51.015.4:[612.352.3:612.14

KEY WORDS: angiotensinogen; blood; estradiol.

The existence of systemic parasexual effects of sex steroids is no longer disputed [3]. It has been shown that estrogens (E) have various metabolic effects on the liver [2, 9, 10], which possesses specific receptors for them [1, 5-8, 10, 14] and, in consequence of this, can be classed as a target organ for E. One process under the control of E is synthesis of the protein angiotensinogen (AG), secreted by the liver [10, 13]; AG is a precursor of angiotensins which participate directly in blood pressure control. It is also known that many pathological (tumors of the female reproductive system, estrogen therapy) and, in particular, physiological states (pregnancy, the menopause, prolonged use of combined contraceptives) in women are accompanied by arterial hypertension. Hence the interest in the study of the role of E in the development of hypertension.

The aim of this investigation was to study the principles governing the effect of estradiol (E_2) in combination with other endocrine factors on the plasma AG level and its dynamics in rats.

EXPERIMENTAL METHOD

Experiments were carried out mainly on mature female (irrespective of the stage of the cycle) and male albino rats of a mixed population. To rule out any effect of endogenous E, mainly ovariectomized females were used. Ovariectomized and hypophysectomized animals were used in the experiments 2-3 weeks after the operation. E_2 (from Serva, West Germany) and testosterone propionate — TP (S. Ordzhonikidze All-Union Pharmaceutical Chemical Research Institute, USSR) were injected subcutaneously in 0.2 ml of propylene-glycol. Control animals received 0.2 ml of solvent. Human somatotrophic hormone (STH) (Kaunas Endocrine Preparations Factory, activity 1 U/mg) was injected subcutaneously in a dose of 200 μg in 0.4 ml physiological saline daily for 5 days. Prolonged injections of the steroids were given every 24 h for 7 days. Each group included 5-6 animals; AG was determined in plasma from each animal. Plasma was obtained by centrifugation of venous blood (2.0 ml blood with 0.1 ml of 2% EDTA solution). AG was converted into angiotensin I (AI) by incubation of 0.05 ml of plasma, diluted 20-30 times, for 3.5 h at 37°C with an excess of renin (0.03 mg in 0.45 ml of 0.1M phosphate buffer, containing 50 mM EDTA, pH 6.5). Rat renin was obtained by the method in [12] (procedure B). To inhibit angiotensinase activity, 0.02 ml of phenylmethylsulfonyl fluoride inhibitor (50 mg in 1.0 ml of absolute ethanol) and 0.48 ml of phosphate buffer were added to the samples. The quantity of AI formed *in vitro* from endogenous AG was measured by radioimmunoassay using the RENC or RENCKT kit (from International CIS, France). Radioactivity was recorded on a gamma-spectrometer: model 1197 from Searle, England, or model LF-9000, from Beckman, USA. The significance of differences was determined by Student's t test.

EXPERIMENTAL RESULTS

Preliminary investigations showed that after a single injection of 500 μg E_2 into female rats the AG concentration rose slowly until 17 h, reached a maximum by 24 h, and fell slowly until 72 h without reaching the control level. Injection of cycloheximide (CH), an inhibitor of protein biosynthesis, into the animals in a dose of 200 μg per rat 30 min before E_2 was

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(Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 3, pp. 342-345, March, 1984. Original article submitted May 18, 1983.

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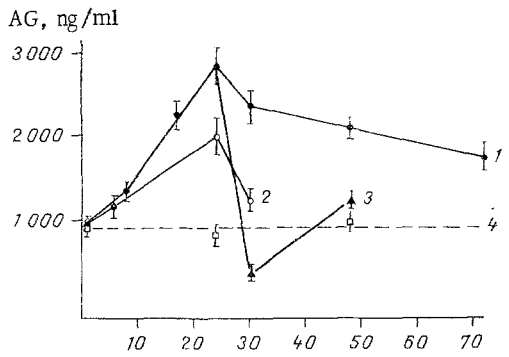

Fig. 1

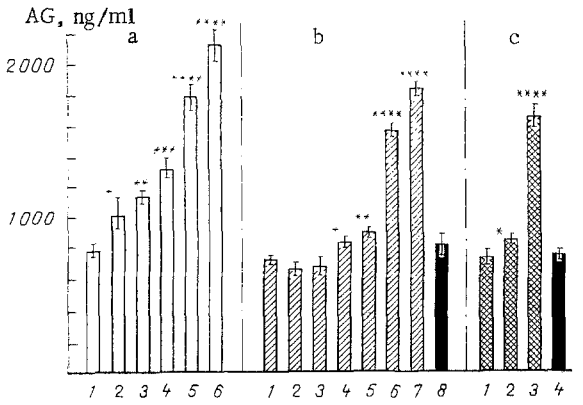

Fig. 2

Fig. 1. Effect of E_2 and CH on plasma AG level of ovariectomized rats (M ± m). 1) E_2 injected; 2) CH injected 30 min before E_2; 3) CH injected 24 h after E_2; 4) control.

Fig. 2. Effect of E_2 and TP on plasma AG level of ovariectomized female and intact male rats (M ± m). a) Single injection of E_2 into female rats 1) control; 2-6) injection of 25, 100, 250, 500, and 1000 μg E_2, respectively (n_1 = 49, n_2 = 15, n_3 = 12, n_4 = 19, n_5 = 39, n_6 = 12); b) repeated injections of E_2 and TP into female rats for 7 days. 1) Control; 2-7) injection of 1, 5, 10, 25, 250, and 500 μg E_2, respectively; 8) injection of 3 mg TP (n_1 = 6; n_2 = 14; n_{3-8} = 6); c) repeated injections of E_2 or TP for 7 days into male rats: 1) control; 2, 3) injection of 10 and 500 μg E_2, respectively; 4) injection of 3 mg TP (n_1 = 6, n_2 = 6, n_3 = 9, n_4 = 6). *P < 0.05, **P < 0.02, ***P < 0.01, ****P < 0.001.

injected, reduced the stimulating effect of the latter by half, whereas injection of CH 24 h after E_2 (i.e., at the height of its effect) caused a sharp fall in the AG level (below the control; Fig. 1). These results indicate, first, that the inducing effect of E_2 is linked with stimulation of AG synthesis in the liver, and second, that AG synthesis takes place actively in the late stages (24 h) after injection of the hormone. Later the plasma AG level of the rats was studied 24 h after a single injection or after the end of repeated injections of E_2 and other hormones.

A single injection of pharmacological doses of E_2 (25, 100, 250, 500, and 1000 μg) into ovariectomized female rats caused a dose-dependent increase in AG formation in the liver (Fig. 2a) . There is evidently a maximal effect for a given dose in this situation; repeated (for 7 days) injection of the same doses of E_2 caused no further increase in the plasma AG concentration (Fig. 2b). Repeated administration of small physiological doses of E_2 (1-5 μg daily for 7 days) had no effect whatever on the AG level, and an increase in the dose to 10 μg daily caused only a very small increase in AG concentration. Some probable reasons for the ineffectiveness of physiological doses of E_2 are its rapid metabolism in the liver and the low sensitivity of the liver to small doses of hormones [10, 13].

It can now be taken as proven that many liver functions are sex-differentiated and are under dual estrogenic—androgenic control [2-4, 9]. Does sexual dimorphism exist in the response of AG biosynthesis to injection of E_2, and can AG formation be stimulated by injection of the male sex hormone testosterone into male and female rats? The results of our experiments shown in Fig. 2c answer this question in the negative. Injection of equal doses of E_2 (10 and 500 μg daily for 7 days) into male and female rats stimulated AG formation in their liver. TP in a dose of 3.0 mg daily for 7 days had no effect either in males or in females. The presence of specific receptors for E in the male rat liver in numbers comparable with their number in females [1, 5] can completely explain the sensitivity of male hepatocytes to the action of E.

The experimental results (Fig. 3a) show that ovariectomy in rats does not affect the plasma AG level. E probably do not play an essential role in maintaining the basal level of AG synthesis in the liver, for in sexually immature female rats also the initial AG level was the same as that in adult animals. However, the effect of large doses of E_2 (250 μg) on immature animals was not demonstrated. It can be tentatively suggested that sensitivity of hepatocytes to the action of E_2 depends on the general endocrine status of the animal, which var-

TABLE 1. Effect of E_2 on Plasma AG Level of Spontaneously Hypertensive (SHR) Rats and Rats of the "Parental" WK Line (M ± m)

Group of animals	Experimental conditions	Number of tests	AG, ng/ml, M ± m	P
Albino rats of mixed population	1. Intact animals	37	854 ± 42	
	2. Intact animals + 10 μg E_2 for 1 day	12	973 ± 78	$P_{1-2} > 0.1$
	3. Ovariectomized animals	6	805 ± 55	$P_{1-3} > 0.1$
	4. Ovariectomized animals + 5 μg E_2 for 7 days	6	864 ± 81	$P_{3-4} > 0.1$
SHR rats	5. Intact animals	12	1051 ± 42	$P_{1-5} < 0.01$
	6. Intact animals + 10 μg E_2 for 1 day	12	1138 ± 102	$P_{5-6} > 0.1$
	7. Ovariectomized animals	6	1351 ± 125	$P_{3-7} < 0.02$
	8. Ovariectomized animals + 5 μg E_2 for 7 days	6	1162 ± 128	$P_{7-8} > 0.1$
WK rats	9. Intact animals	6	1148 ± 38	$P_{1-9} < 0.01$
	10. Intact animals + 10 μg E_2 for 1 day	10	1434 ± 77	$P_{9-10} < 0.01$
	11. Ovariectomized animals + 5 μg E_2 for 7 days	6	1442 ± 40	$P_{9-11} < 0.02$

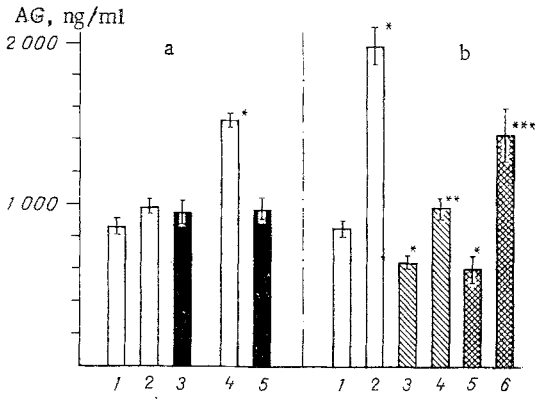

Fig. 3. Effect of various endocrine factors on plasma AG level of female rats (M ± m). a) Effect of ovariectomy and puberty on plasma AG level and its sensitivity to E_2: 1) sexually mature rats; 2) ovariectomized animals 3 weeks after operation; 3) sexually immature rats; 4) adult rats after single injection of 250 μg E_2; 5) immature rats after a single injection of 250 μg E_2 ($n_1 = 37$, $n_2 = 49$, $n_3 = 13$, $n_4 = 13$, $n_5 = 6$). *$P < 0.01$; b) effect of hypophysectomy and STH on realization of effect of E_2. STH injected in a dose of 200 μg daily for 5 days, E_2 as a single dose of 500 μg 24 h after end of STH injection. Blood taken 24 h after injection of E_2 or end of injection of STH. 1) Intact females; 2) the same animals after injection of E_2; 3) hypophysectomized females 2 weeks after operation; 4) the same animals after injection of E_2; 5) hypophysectomized females after injections of STH; 6) the same animals after injection of E_2 ($n_1 = 37$, $n_2 = 26$, $n_3 = 39$, $n_4 = 26$, $n_{5,6} = 12$). *$P < 0.001$ compared with group 1, **$P < 0.001$ compared with group 2, ***$P < 0.02$ compared with groups 2 and 4.

ies during sexual maturation. A certain hepatotrophic factor of the pituitary, "feminotrophin," which apparently participates in the realization of the effect of sex hormones on the liver [9, 11], may perhaps play an essential role in this situation. The most likely candidate for the role of "feminotrophin" is STH [9]. It was therefore interesting to study the effect of hypophysectomy on the AG level and its sensitivity to the action of E_2. The data showed that hypophysectomy, which lowers the plasma AG level in female rats only slightly, sharply de-

pressed the stimulating effect of E_2 (Fig. 3b). This suggests that pituitary factors have a permissive, and not a mediating function in the hepatotrophic action of E. The mechanism of this permissive effect of pituitary factors may be regulation of the level of hepatic estrogen receptors.

The sensitivity of hepatocytes to the stimulating action of E is also determined by a genetic factor (Table 1). For instance, a high plasma AG concentration is observed in rats of the "parental" Wistar-Kyoto (WK) line, which basically have a normal blood pressure, and which give rise to the SHR line of rats with spontaneous genetic hypertension. It is particularly interesting that increased sensitivity of AG production to E_2 is found in rats. The effect is observed after injection of as little as a single dose of 10 μg or 5 μg daily for 7 days. Neither normal rats nor rats with marked genetic arterial hypertension, whose plasma AG level is raised de novo, react to these doses.

The role of each of the hypothetical hepatotrophic factors, their mutual effect, and the direct mechanism of modulation of hepatocyte reactivity to the action of E, which stimulates AG synthesis, and also the role of estrogen-sensitive metabolic processes in the liver in the realization of the sex-dependent character of certain types of cardiovascular and other pathology are not yet sufficiently clear and require further study.

The SHR and WK rats were generously provided by the Laboratory of Pathological Physiology, Institute of Pediatrics, Academy of Medical Sciences of the USSR (Head, Professor Kh. M. Markov).

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