EFFECT OF ALCOHOL ON SEX STEROID RECEPTOR LEVELS IN THE HYPOTHALAMUS AND PITUITARY OF MALE RATS

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Alcohol has marked ability to modify activity of the endocrine system. Among the endocrinopathies caused by administration of alcohol, one of the most important is damage to the hypothalamo-hypophyseo-gonads system, although it is not known whether alcohol exerts its toxic effect on all or on one of its components. Investigations have shown that ethanol can cause a disturbance of the mechanism of negative feedback between gonadotrophins and sex hormones in male rats [6, 10].

The writers showed previously that andogenic and estrogenic receptors in the hypothalamus and pituitary of male rats may play an important role in gonadotrophyn release by a negative feedback mechanism [4, 5, 7]. Consequently, uncoupling of the feedback mechanism between the gonads and pituitary, due to the action of ethanol, may be the result of a change in the number of specific sites, binding sex hormones, in the hypothalamus and pituitary.

It was accordingly decided to study receptor-bound testosterone and extradiol in the hypothalamus and pituitary of sexually mature male rats on a model of experimental alcoholism (formation of physical dependence by the method of intensive alcoholization).

EXPERIMENTAL METHOD

Noninbred male rats weighing 200-250 g were used. Physical dependence on alcohol was formed by continuous intoxication with 25% ethanol. To develop a withdrawal reaction in the animals, a direct sign of physical dependence on ethanol, the method of intragastric incubation was used [11], with certain modifications [1]. For 5 days, twice a day (at 10 a.m. and 4 a.m.) the animals were given a 25% solution of ethanol in a dose of 7.5 g/kg. Control animals were given water. The rats were decapitated on the 5th day, 1 h after the last injection of ethanol – at the peak of alcoholic narcosis (group 1) and on the 6th day, 20 h after the final injection of ethanol, when manifestations of a withdrawal reaction were observed (group 2). The body weight of the animals after 5 days of the experiment was reduced by 10-15%. Hypothalamic fragments (preoptic-anterior hypothalamic region – PR, and region of the mediobasal hypothalamus – MBH) and poled adenohypophyses from 40 or 50 animals, were homogenized and centrifuged, as described previously, to obtain cytoplasmic and nuclear fractions [3]. 1,2,6,7-^{3}H-Testosterone and 2,4,6,7-^{3}H-estradiol, with specific radioactivity of 85-120 Ci/mmole were used as the labeled hormones.

The conditions of incubation of the labeled steroids with the cytoplasmic and nuclear fractions of the pituitary and hypothalamus and the method of calculating the number of specific binding sites were the same as in the work published previously [4]. The number of binding sites in the cytosol was calculated in femtomoles/mg protein, and in the nuclei, in femtomoles per milligram DNA. Concentrations of luteinizing (LH) and follicle-stimulating (FSH) hormones in the blood were determined in the control and experimental animals by radioimmunoassay [2], and the blood testosterone level was determined with the aid of a kit for radioimmunoassay of testosterone (the Steron-T-^{3}H).

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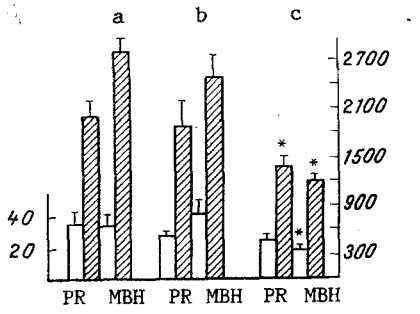

Fig. 1

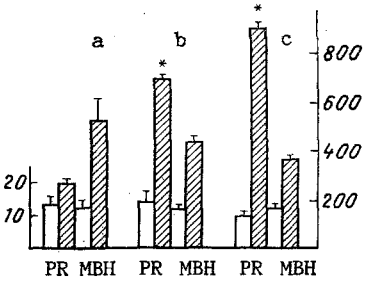

Fig. 2

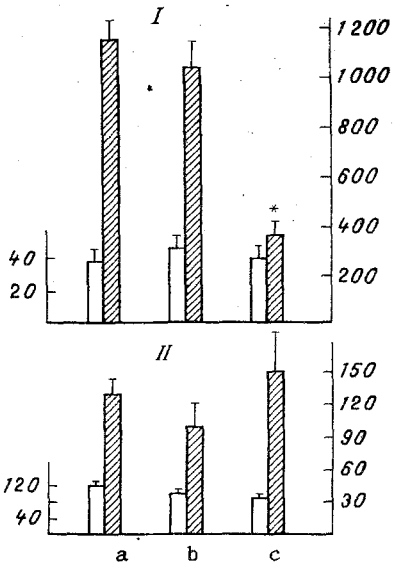

Fig. 3

Fig. 1. Changes in concentration of cytoplasmic and nuclear testosterone receptors in hypothalamus of control male rats (a), and rats in a state of alcoholic narcosis (b) and withdrawal (c). Ordinate: on left – bound ^{3}H-hormone in cytoplasmic fraction (in fmoles/mg protein), on right – bound ^{3}H-hormone in nuclear fraction (in fmoles/mg DNA). Unshaded columns – cytoplasmic, shaded – nuclear receptors. *$p < 0.05$ compared with control.

Fig. 2. Changes in concentration of cytoplasmic and nuclear estradiol receptors in hypothalamus of control rats (a) and rats in a state of alcoholic narcosis (b) and withdrawal (c). Legend as to Fig. 1.

Fig. 3. Changes in concentration of cytoplasmic and nuclear testosterone (I) and estradiol (II) receptors in pituitary of control rats (a) and rats in states of alcoholic narcosis (b) and withdrawal (c). Remainder of legend as to Fig. 1.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that the blood testosterone level of the rats was significantly lower on the 5th day of ethanol administration, and fell lower still during the withdrawal reaction. Meanwhile the blood LH and FSH levels of the alcoholized rats were the same as in the control. Consequently, during experimental alcoholization the gonadotropin level remains unchanged, and no adequate release of LH and FSH is observed in response to the sharp decline in the steroid-synthesizing function of the gonads. This is evidence of disturbed functions of the pituitary-gonad system.

Injection of ethanol did not change the number of testosterone-binding sites in either the cytoplasmic or the nuclear fractions of PR and MBH in the rats of group 1. In rats in a state of abstinence, there was a marked fall in the concentration of androgenic receptors in the cytoplasmic fraction of MBH and the nuclear fraction of PR and MBH (Fig. 1). Meanwhile a significant increase in the number of estradiol-binding sites was observed in the nuclear fraction of PR in rats of both group 1 and group 2. The concentration of estrogenic receptors in MBH was unchanged (Fig. 2).

Unlike the hypothalamus, changes in the adenohypophysis involved only androgenic receptors: a sharp decrease was observed in the number of testosterone-binding sites in the nuclear fraction of the pituitary of the rats in a state of withdrawal (Fig. 3).

The negative feedback action of sex hormones on the hypothalamic-pituitary-gonads system in male rats is known to be mediated at the MBH level, and the high level of nuclear testosterone receptors which we observed in MBH of intact males is evidence that androgenic receptors participate in this mechanism. A high concentration of nuclear androgenic receptors was found in PR on intact rats also. Since PR is responsible for the manifestations of male sexual behavior, and is particularly sensitive to the action of testosterone, the presence of a high concentration of androgenic receptors in this region is evidence of the great importance of the receptor component in adrogen-dependent motivation of sexual behavior.

TABLE 1. Effect of Repeated Injections of Ethanol on Blood Serum Testosterone, LH, and FSH Levels of Male Rats (M ± m)

Group of rats	Testosterone, ng/ml	LH, ng/ml	FSH, ng/ml
Control	0,95±0,10 (6)	102,7±7,7	(6) 98,0±7,5 (7)
1.	0,71±0,02* (6)	119,3±6,6	(11) 89,4±4,0 (15)
2.	0,43±0,03* (7)	102,5±0,5	(17) 88,8±3,4 (19)

Legend. Asterisk indicates significant differences from control. Number of determinations shown in parentheses.

In rats in a state of alcoholic narcosis, despite the fall of the blood testosterone level, no appreciable changes were found in the concentration of androgenic receptors in either PR or MBH. The fall of the testosterone level may perhaps not change the sensitivity of the steroid-sensitive neurons of the hypothalamus to the action of the hormone. The blood testosterone level in male rats in a state of withdrawal was sharply reduced and a decrease was observed in the number of nuclear testosterone-binding sites in MSBH and PR, indicating a decrease in sensitivity of the androgen-concentrating neurons in these regions to the action of testosterone. This may evidently be the cause of the change in sexual behavior of male rats during the alcohol withdrawal reaction.

The levels of nuclear estrogenic receptors in PR was 3.5 times higher in male rats in a state of withdrawal. Analysis of data in the literature showed that a raised level of estradiol and, in particular, of estrone in the blood plasma of alcoholics (both humans and animals) can be attributed mainly to a disturbance of steroid metabolism in the liver, with an increase in biotransformation of androgens into estrogens [8, 9]. In the opinion of the authors cited, these processes are evidently among the leading causes of feminization observed in alcoholized male individuals. It is considered [8] that the sharp increase (by 50%) in synthetic estrogen binding in the liver cytosol of male, but not female rats, starting with the 7th day of ethanol consumption by the rats, as a component of the liquid diet, can explain the characteristic feminization of males consuming ethanol for a long time. It can be postulated on the basis of the results that during repeated injections of ethanol intensification of the aromatization of androgens into estrogens is observed also in the hypothalamus of male rats, and the sharp increase in specific binding of estradiol with nuclear receptors of PR can evidently also explain the feminization of alcoholized males.

The level of nuclear androgenic receptors in the adenohypophysis, which also are involved in the regulation of gonadotrophin release by a negative feedback mechanism, is reduced in males during the alcohol withdrawal reaction. Consequently, the ability of gonadotrophs to bind testosterone is reduced and, in turn, this may modify the response to releasing hormone. Data in the literature on this matter are contradictory, but some workers have discovered inadequate release of LH in response to injection of H-releasing hormone [12].

By contrast with the hypothalamus, the number of estradiol-binding sites in the pituitary of alcoholized males was unchanged. Previous investigations showed that the inhibitory action of estradiol on LH secretion in male rats is not mediated through estrogenic receptors of the pituitary, and they evidently do not play any physiological role in processes of regulation [4].

The results of this investigation thus indicate that repeated injections of alcohol lead to a testosterone deficiency in the blood, and a deficiency of androgenic receptors in MBH and the adenohypophysis; these phenomena may perhaps lead to uncoupling of the mechanism of negative feedback between the gonads and pituitary.

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EFFECT OF OPIATE RECEPTOR AGONISTS AND ANTAGONISTS ON MATERNAL AGGRESSION IN RATS

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Convincing results indicating involvement of the opiate systems of the brain in the regulation of affective behavior, of intraspecific attachment and sociability have recently been obtained [1, 3-5]. Data on the role of the opiate systems in integration of maternal behavior and, in particular, aggression in lactating females, remain very limited. Accordingly, the aim of the present investigation was to study the effects of opiate-positive and negative drugs on the structure of behavior of lactating females relative to a strange male and offspring.

EXPERIMENTAL METHOD

Experiments were carried out on 54 lactating female rats weighing 250-350 g during the two weeks after giving birth. Intraspecific behavior of the mothers was analyzed 20 min after intraperitoneal injections of physiological saline or the appropriate drug. For this purpose, a strange male was placed in the cage in which the female and her offspring were kept constantly, and the behavior of the female toward the male and the offspring was assessed for a period of 5 min. The data were recorded and analyzed statistically by methods of computerized etiologic pharmacology [2, 3], using the "Étograf-ÉVM" complex. The behavior of the lactating females was described by means of a discrete stationary mathematical model [3]. Matrices of statistical probabilities of diadic transitions of behavioral elements were represented by directional graphs. Nonparametric tests were used for rapid analysis of the effects of the drugs. Morphine, buprenorphine, tifluadom ("Sandoz"), and bremazocine ("Sandoz") were used as agonists of opiate systems, and naloxone and naltrexone (both from "Endo Laboratories") as antagonists.

EXPERIMENTAL RESULTS

The μ-agonist morphine and also buprenorphine (Table 1) induced a dose-dependent reduction of aggression of the female toward the male. Buprenorphine facilitated individual behavior and nonagonistic forms of intraspecific interactions (such as sociability toward the male, active and passive contacts with the offspring) a little. Buprenorphine changed not only the probability of appearance of elements of intraspecific behavior, but also their order and interdependence, i.e., the program of behavior, as was shown by computer analysis of the behavior graph (Fig. 1). Elements of aggressive and ambivalent interaction (attacks,

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