Effects of 17β-Estradiol and Its Isomer 17α-Estradiol on Learning in Rats with Chronic Cholinergic Deficiency in the Brain

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It was shown for the first time that estrogens 17β- and 17α-estradiols compensate impaired cognitive functions in rats with partial chronic deprivation of cholinergic functions in the central nervous system induced by intracerebral administration of selective cholinergic neurotoxin AF64A. 17β-Estradiol produced strong dose-dependent changes in the weights of hormone-sensitive endocrine glands, while 17α-estradiol did not affect the weight of the gonads and slightly influenced (in high concentration) the weights of the adrenal glands and thymus. The positive effects of exogenous 17β- and 17α-estradiols on cognitive functions are due to their antioxidant properties, rather than due to specific action on hormone-sensitive endocrine glands.

Key Words: *17β- and 17α-estradiosl; neurotoxin AF64A; active avoidance; rats*

Endocrine dysfunction and Alzheimer's disease (AD) characterized by degeneration of acetylcholine neurons in the brain are prevalent in elderly people. It was shown that the increase in blood estrogen level is associated with low risk of AD [8]. Estrogens improve memory in laboratory animals with impaired energy metabolism [10] and brain ischemia [3]. It was shown that 17β-estradiol (17β-ED) compensates working memory disturbances after scopolamine blockade of acetylcholine receptors in the brain [4,6]. It is interesting to evaluate whether or not estrogens affect cognitive functions in animals, whose physiological state is similar to that in AD. Chronic deprivation of cholinergic functions in the central nervous system produced by intracerebral administration of neurotoxic acetylcholine analogue 1-ethyl-1-(2-hydroxyethyl)-ethylenimine (AF64A) is a common model of AD [5,7,11,15]. In this case, degeneration of acetylcholine nerve endings is accompanied by long-term oxidative stress in damaged structures, in particular in the cortex and hippocampus [7]. It should be emphasized that activation of lipid peroxidation in the brain is typical of patients with AD [12].

Here we studied the effects of 17β-ED and its isomer 17α-ED on learning in rats with chronic deprivation of cholinergic functions induced by intracerebral injection of AF64A.

MATERIALS AND METHODS

Experiments were performed in winter Wistar rats weighing 180-200 g were obtained from the Stolbovaya nursery. Castrated animals received subcutaneous injections of 17β-ED or 17α-ED (Sigma) in oil for 7 days before and 10-12 days after administration of AF64A. The animals of two control groups were injected with oil. AF64A was prepared from AF64 (Bachem) immediately before injection (Fisher's method) [5] and diluted with artificial cerebrospinal fluid (CSF). Under ether anesthesia, treated rats and group 1 control animals were stereotaxically injected (intracerebroventricularly) with 3 nmol AF64A in 3 μl CSF;

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group 2 control rats received 3 μl CSF. Ten-twelve days postinjection, the rats were trained active avoidance task in a shuttle box [11]. The criterion of conditioning in two-way avoidance paradigm was rat transition to the dark compartment after light signal (conditioned run) preceding electric shock. The training course consisted of 35 presentations, and memory consolidation was assessed on the next day by the same procedure. We recorded the mean numbers of conditioned runs in blocks of 5 consecutive trials for the last 15 stimulus presentations on the 1st day of conditioning and for the first 15 stimulus presentations on the 2nd day. The rats were then decapitated, and hormone-sensitive organs were isolated and weighted. Each of 4 independent experiments was conducted on 30-36 rats. The results were analyzed by one-way ANOVA and Newman—Keuls test.

RESULTS

It was shown that castration of rats for correction of their hormonal status does not change the reaction to AF64A compared to that of intact animals [11]. Neurotoxin AF64A impaired learning and retrieval (Table 1), but practically did not affect the weight of hormone-sensitive organs in castrated animals except for a considerable, but statistically insignificant decrease in the thymus weight (Table 2). Systemic administration of 17α-ED and 17β-ED improved (to a different extent) cognitive functions in rats treated with AF64A. 17β-ED in a dose of 40 μg/kg improved learning and retrieval of memory traces on day 2, but did not affect conditioning in a dose of 4 μg/kg. 17α-ED in both doses improved learning and memory retrieval and, therefore, was a more potent stimulator of cognitive functions than 17β-ED (Table 1). 17β-ED dose-dependently increased the weights of the prostate, seminal vesicles, and adrenal glands, but decreased thymus weight. 17α-ED did not affect the weight of the adrenal glands. 17α-ED in a dose of 40 μg/kg increased the weight of the adrenal glands and decreased the weight of the thymus; in a dose of 4 μg/kg this hormone was ineffective (Table 2). These data indicate that the effects of 17α-ED and 17β-ED on the brain are realized via various mechanisms.

Previous studies showed that preliminary systemic injections of 17β-ED attenuate necrotic changes during ischemic damage [3]. In our experiments, improvement of learning is probably due enhanced resistance of the nervous tissue to AF64A and/or activation of compensatory and regenerative processes by exogenous estrogens. Probably, one of the mechanisms of estrogen effects on learning is their direct influence on transcription during the interaction with nuclear receptors for estrogens in the brain. However, estrogen receptor density in the hippocampus and cortex is relatively low [9]. 17α-ED is characterized by lower affinity for estrogen receptors and less markedly activates transcription than 17β-ED [1]. Hence, it is unlikely that the neuroprotective effect of estradiols is related to modulation of transcription mediated via nuclear receptors. There are no data ex-

TABLE 1. Two-Way Avoidance Conditioning (% of Correct Responses) in Rats with Partial Deprivation of Cholinergic Functions (*M±m*)

Day	Control (CSF)	AF64A	AF64A+17β-ED, μg/kg		AF64A+17α-ED, μg/kg	
			40	4	40	4
1st	91.2±1.8*	51.6±4.2	84.6±3.8*	48.0±12.4	77.4±6.8**	74.0±9.8**
2nd	86.0±2.2*	42.0±4.2	82.0±4.8*	53.4±10.4	62.6±10.0*	82.6±9.6**

TABLE 2. Changes in the Weight of Hormone-Sensitive Organs in Castrated Rats after Systemic Injections of 17β- and 17α-ED (% of Control, *M±m*)

Organ	Control (CSF+ solvent)	AF64A+ solvent	AF64A+17β-ED, μg/kg		AF64A+17α-ED, μg/kg	
			40	4	40	4
Prostate gland	100.0±4.7	103.4±5.2	158.1±11.1*+	129.0±5.4*++	104.5±5.1	104.7±5.4
Seminal vesicles	100.0±4.3	99.2±4.4	152.1±18.6*+	127.5±5.4*++	102.5±4.3	95.3±6.0
Adrenal glands	100.0±1.9	97.2±3.2	113.4±3.8*++	128.7±5.8*+	127.7±6.0*+	100.9±3.5
Thymus	100.0±3.9	91.5±4.6	69.7±4.7*++	76.6±6.4*++	81.6±3.9*++	98.9±6.8
Spleen	100.0±2.2	105.8±4.2	90.7±3.0	92.5±5.7	95.6±5.6	107.8±8.2

Note. *$p<0.001$ and **$p<0.01$ compared to the control, +$p<0.001$ and ++$p<0.01$ compared to AF64A.

plaining the more pronounced effect of 17α-ED on learning (compared to that of 17β-ED) by other mechanisms of neuroprotective effects of estrogens, including their influence on mitochondria or modulation of binding of neuromediators to receptors on cell membranes [14]. Neuroprotective effects of estrogens can be realized via cell protection due to their considerable antioxidant properties [2]. The influence of 17β-ED on cognitive functions was most extensively studied. The protective properties of this hormone were demonstrated in various models of brain disorders [3,4,6,10]. Hence, its effects are realized via the same antioxidant mechanism. 17α-ED is a more potent antioxidant than 17β-ED [13]. 17α-ED improves learning in AF64A-treated rats in a dose much lower than that of 17β-ED. The data suggest that the protective effects of these estrogens are realized via the antioxidant mechanism.

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