Effects of Synthetic and Plant-Derived Selective Modulators of Estrogen Receptors on Depression-Like Behavior of Female Rats

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Effects of chronic (for 14 days) intramuscular injections of tamoxifen (10 mg/kg), klimadynon (Cimicifuga extract; 20 mg/kg), and genistein (10 mg/kg) on depression-like state were studied in female rats under conditions of natural fluctuations of blood estrogen levels and ovariectomy. Chronic tamoxifen caused a pronounced depression, while chronic klimadynon and genistein exhibited a pronounced antidepressant effect in intact and ovariectomized rats.

Key Words: *tamoxifen; klimadynon; genistein; Porsolt test; depression-like behavior*

It is well known that in addition to their effect on the reproductive system, estrogens modulate the psychoneurological status of females [4,11]. Depression is one of the symptoms of premenstrual tension and menopause [5]. Recent studies indicate that replacement hormone therapy improves patients' quality of life, eliminating panic disorders, anxiety, weepiness, fear, depressive moods [12]. However, clinical findings indicate that hormone replacement therapy is fraught with a high risk of early stroke and venous thromboembolism, while the risk of breast cancer increases with prolongation of therapy [2]. This necessitates the search for alternative therapeutic methods. An alternative evaluated in our study is selective modulators of estrogen receptors (SMER), a new class of drugs for patients with contraindications precluding hormone replacement therapy or unwilling to use it. Previously SMER were regarded as antiestrogens, while today we know that this assumption is erroneous. The term SMER was designated to denote compounds which, in contrast to "pure" estrogen agonists and antagonists, were characterized by mixed and selective mode of action, depending on damaged tissue.

Synthetic drugs tamoxifen and raloxifene are best studied. Drugs of plant origin have been studied in recent years. Climadinon (*Cimicifuga racemosa*) is now widely used for correction of menopausal vasomotor symptoms.

High selective activity of these drugs towards estrogen-sensitive tissues suggested evaluating their effect on the psychoemotional status under conditions of estrogen deficiency and natural fluctuations of estrogen levels throughout the estrous cycle.

We compared the effects of selective modulators of estrogen receptors of synthetic and plant origin on depression-like behavior of intact and ovariectomized (OE) female rats.

MATERIALS AND METHODS

The study was carried out on 80 adult female Wistar rats (220-250 g) from Rappolovo Breeding Center. The animals were kept at natural illumination, standard temperature, and ration with free ac-

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cess to water and food. The studies were carried out in the morning (9.00-12.00). For behavioral tests, the animals were divided into groups of 8-10 rats: 1) intact females injected with saline (control group 1); 2) intact females injected with 0.5 μg 17β-estradiol (Sigma); 3) intact females injected with tamoxifen (10 mg/kg; Orion); 4) intact females injected with klimadynon (40 mg/kg; Bionorica); 5) intact females injected with genistein (10 mg/kg; Sigma); 6) OE females injected with oil solvent (control group 2); 7) OE females injected with 0.5 μg 17β-estradiol (Sigma); 8) OE females injected with tamoxifen (10 mg/kg; Orion); 9) OE females injected with klimadynon (40 mg/kg; Bionorica); and 10) OE females injected with genistein (10 mg/kg; Sigma). All drugs, 17β-estradiol, and oil solvent were injected intramuscularly daily for 14 days.

The ovaries were removed by the common method and drug injections were started after 2 weeks. Females in the diestrus phase served as controls, because this stage of the cycle is characterized by balanced hormone status.

The severity of depression-like status of animals was evaluated by the duration of immobilization in Porsolt test [1].

The data were statistically processed using one-way dispersion analysis (ANOVA; post-hoc: Tukey test) at $p<0.05$.

RESULTS

Porsolt test showed that the duration of immobilization of intact females in the estrus and metestrus phases, characterized by low blood estrogen levels, was lower than in females in the diestrus and proestrus phases characterized by medium and high concentrations of natural estrogens in the blood, respectively (Fig. 1). Chronic treatment of intact females by 17β-estradiol disturbed the estrous cycle. On day 3 of the drug injections all animals were in the estrus phase. Porsolt test showed that 17β-estradiol significantly prolonged the immobilization period ($p<0.05$) in comparison with intact females in the same phase. Importantly that by the moment of the first injection of tamoxifen the group of intact animals was balanced so that the same number of females were in each phase of the estrous cycle ($n=9$). On days 7-10 of tamoxifen treatment, the ovarian cycle was disordered, the estrus phase being absent. The immobilization period of intact females, treated by tamoxifen, was significantly shorter during diestrus and metestrus phases ($p<0.05$) in comparison with the control females in the same phase. However, the duration of immobilization in the proestrus phase was significantly longer than in control group 1 (proestrus). Chronic injections of klimadynon to intact females reduced significantly the immobilization period ($p<0.05$) during diestrus and metestrus phases and increased it in the proestrus and estrus phases in comparison with control females with the same phases of the cycle. Chronic treatment of intact females by genistein led to significant shortening of immobilization period during all cycle phases in comparison with control group 1.

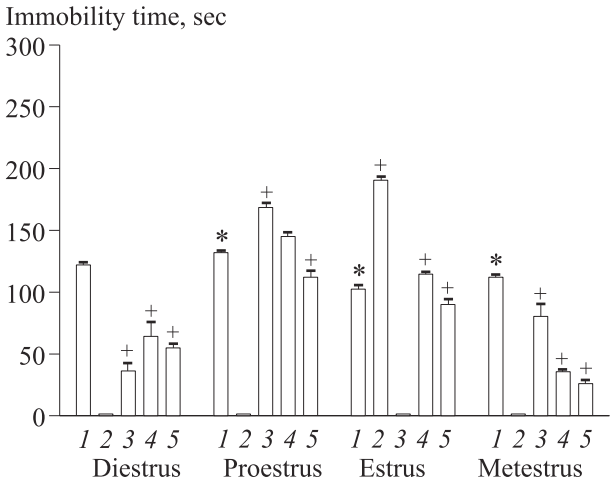

Fig. 1. Effect of chronic tamoxifen, klimadynon, and genistein treatment on depression-like behavior of intact rats in Porsolt test. *1*) intact females (control 1); *2*) intact females+17β-estradiol; *3*) intact females+tamoxifen; *4*) intact females+klimadynon; *5*) intact females+genistein. $p<0.05$ *between cell cycle phases in the control group, +in comparison with control group 1.

The duration of immobilization increased significantly under conditions of estrogen deficiency in comparison with intact females (diestrus) (Fig. 2).

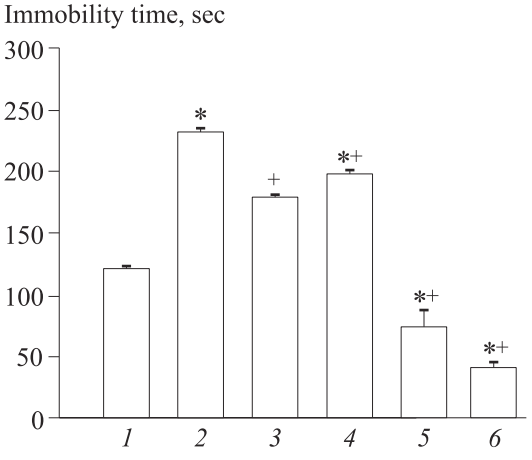

Fig. 2. Effect of chronic tamoxifen, klimadynon, and genistein treatment on depression-like behavior of OE-rats in Porsolt test. *1*) intact females (diestrus; control 1); *2*) OE females (control 2); *3*) OE females+17β-estradiol; *4*) OE females+tamoxifen; *5*) OE females+klimadynon; *6*) OE females+genistein. $p<0.05$ compared to: *control group 1, +control group 2.

Replacement hormone therapy of OE females by 17β-estradiol resulted in a significant reduction of immobilization period ($p<0.05$) in comparison with control OE females. The duration of immobilization in OE females receiving chronic tamoxifen treatment decrerased negligibly in comparison with OE females, but this parameter was higher than in the group receiving 17β-estradiol. Chronic klimadynon and genistein treatment of OE females caused a significant ($p<0.05$) reduction of immobilization period in comparison with control groups 1 and 2 and with the group receiving 17β-estradiol therapy.

The results indicate that 17β-estradiol and synthetic drug tamoxifen disorder the ovarian cycle in intact females. High blood concentration of estradiol blocks the negative feedback in the hypothalamic—pituitary—ovarian system, which leads to permanent estrous in intact females. In addition, high blood level of estradiol promotes the development of depressive effect in rats, which is presumably related to inhibition of serotonin synthesis and suppression of its reuptake in brain structures. The relationship between serotonin content in the hypothalamus and sex cycle phases is a known fact [8]. The minimum content of serotonin corresponds to the ovulation, characterized by high level of estrogens (late proestrus, onset of estrus) [8]. Tamoxifen also modulates the functional activity of the hypothalamic—pituitary—ovarian system. As a highly sensitive positive ligand for estrogen receptor (ER) α-form, tamoxifen causes priming of gonadotropin releasing hormone [9], reduces target cell sensitivity to lutropin, which leads to inhibition and suppression of secretion of luteinizing hormone (LH) [9], the main stimulator of ovulation and corpus lutenum formation. Hence, the absence of estrus is presumably linked with suppression of the preovulatory release of LH and subsequent ovulation, as a result of which the corpus luteum formation is disturbed. At the same time, it is obvious that behavioral effects of tamoxifen depend on blood concentration of endogenous estrogens. The effect of tamoxifen is antidepressant in the presence of low (metestrus) and medium (diestrus) estradiol concentrations in the blood. Presumably, it is due to tamoxifen capacity to stimulate the expression of α-ER and increase their affinity for their ligands [9]. More severe depression-like state during the proestrus phase (high blood estrogen level) is presumably caused by tamoxifen stimulation of the modulating effect of the estrogens on functional activity of serotoninergic system. In addition, it is known that tamoxifen blocks β-ER, through which the antidepressant effect of estradiol is realized [11]. In contrast to synthetic SMER, klimadynon and genistein do not modulate the phase pattern of the sexual cycle in females. Antidepressant effect of klimadynon in the diestrus and metestrus phases is explained by very low concentration of endogenous estrogens, due to which this drug exhibits higher affinity for ER. In addition, phytoestrogens increase the expression of α- and β-ER mRNA in the hypothalamus [7], which also promotes manifestations of klimadynon effects. The absence of antidepressant effect of klimadynon in the proestrus and estrus phases can be due to higher affinity of ER endogenous ligand in the brain (estradiol), which leads to attenuation of the drug effect. In addition, ballast substances present in klimadynon can inhibit the release of phytoestrogens and the intensity of their effects. On the other hand, genistein, a phytoestrogen free from ballast and auxiliary substances, exhibited a pronounced antidepressant effect during all phases of the cycle.

The development of a manifest depressive condition in OE females in comparison with intact animals and antidepressant effect of replacement 17β-estradiol therapy in OE females were observed. According to published data, increased expression of 5-HT_{1A}/5-HT_{2A} receptor genes in OE rats receiving chronic 17β-estradiol correlates with decreased expression of 17β-ER gene, on the one hand, and with antidepressant effect of estradiol, on the other [3]. However, injection of estradiol as replacement monotherapy to OE rats is insufficient to completely eliminate depression-like behavior, and therefore, the search for alternative therapeutic methods is an important problem. Chronic injection of tamoxifen to OE females leads to the development of depression. The depressive effect of this drug can be explained by its capacity to modulate the conformation of one or both ER types, through which estrogens indirectly increase the expression of 5-HT_{2A} receptor gene and of serotonin return transport gene in the suture nuclei, and increase the density of 5-HT_{2A} receptors in the forebrain [10]. A pronounced antidepressant effect of klimadynon and genistein in OE females confirms the hypothesis according to which phytoestrogens under conditions of endogenous estrogen deficiency are highly sensitive ER agonists for receptors located in the hypothalamus, hippocampus, and other limbic structures. In addition, a long period of estrogen deficiency caused by ovariectomy inhibits ER capacity to bind ligands [6], while replacement phytoestrogen therapy increases activity of ER ligand site [7], which can explan more pronounced antidepressant effect of klimadynon and genistein.

Hence, the findings indicate the possibility of a new approach to drug therapy of affective disor-

ders in ovarian hypofunction and in the reproductive age in natural fluctuations in estrogen levels.

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