COMPARATIVE AND ONTOGENIC PHYSIOLOGY

**Estrous Cycle, Folliculogenesis, and Brain Catecholamines after Stimulation of the Sexual System by Choriogonadotropin in Female Minks Selected for Behavior**

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**Abstract**—The content of hypothalamic dopamine (DA) and noradrenaline (NA) is determined and ovary function (folliculogenesis) in various states of estrous cycle is studied in non-selected (control) immature (7-month old) standard female minks and in similar females of aggressive and quiet behavioral types (the 18th generation of selection) in norm and at the 7th day after single intramuscular injections of choriionic gonadotropin (CG). At the 7th day after the CG administrations, the control females were distributed by their estrous cycle state as follows: anestrus (A)—47.2%, anestrus-proestrus (AP)—10.0%, proestrus (P)—8.5%, proestrus-estrus (PE)—14.3%, and estrus (E)—20.0%. In the female minks selected for behavior, there was a significant increase of percentage of estrous females: to 84.6% in the aggressive ($p < 0.001$) and to 58.4% in the quiet type animals ($p < 0.01$). The number of growing and maturing (at the early stage) follicles in intact females of aggressive behavior was lower than in control and in females of the quiet type. However, the number of Graafian vesicles in ovaries of aggressive and quiet females did not differ and exceeded control. The stimulation by CG enhanced the differences between the ovaries of selected animals. In control females at the 7th day after CG injections there was a tendency for a decrease of the DA and NA content with development of estrus (A → AP → P). The hypothalamic DA concentration was higher in intact females of the aggressive type than in the quiet females ($p < 0.05$). In animals of the aggressive type, stimulation of the sex system decreased the DA level. No correlation between sexual function and DA and NA levels was found in females of the quiet type. However, differential analysis of NA in these females with different reactions of vaginal epithelium to CG stimulation (A, AP vs P, PE, E) indicates correlation of catecholamine level with sexual function. Thus, selection of minks for aggressive behavioral type produces an increase of the hypothalamic catecholamine level, which, in turn, is one of factors inhibiting sexual function.

**INTRODUCTION**

In studies on foxes [1], rats [2], and minks [3] an evidence for significant changes of the estrous cyclicity has been obtained in long selection experiments on elimination of defensive behavior with respect to human [3, 4]. The mammalian estrous cycle reflects complex temporary and functional interrelations of many structures. Coordination of the estrous cycle processes is largely provided by action of hormones as well as of neurohormones and neurotransmitters. Despite marked achievements in the field of neurohormonal and hormonal regulation of the estrous cycle, many aspects of this problem are studied insufficiently. Most investigations were carried out on laboratory animals; meanwhile use of non-traditional objects allows studying species peculiarities and revealing general fundamental regularities.

One of the key issues in determination of behav-
ior and regulation of reproduction is the state of the brain catecholamine system [5]. It is to be noted that hypothalamus, in which switching of nerve impulses to chains controlling secretion of pituitary gonadotropic hormones occurs [5, 6], is among the brain parts, in which prominent changes in the transmitter metabolism have been found during selection for behavior [5, 6]. Complex studies of the catecholamine content at different states of the sexual system in animals with contrast behavioral patterns can reveal new aspects of association of behavior with reproductive function.

A peculiarity of the mink sexual system allows, on one hand, inducing the female sexual cycle by chorionic gonadotropin (CG) outside reproductive period and, on the other hand, determining parameters of the subsequent folliculogenesis wave [7]; this makes this system unique for studying relations between the neuroendocrine system and sexual function.

The goal of the present work was to determine parameters of the estrous cycle and folliculogenesis as well as content of dopamine (DA) and noradrenaline (NA) in hypothalamus after stimulation with CG in females of aggressive and quiet types of behavior.

MATERIALS AND METHODS

Objects of study were immature, 7-month old, standard females of non-selected (industrial) and selected for behavior (the 18th generation) mink populations. The selection for behavior was performed by the Catch test method that consists in that an experimenter opened the animal cage door and tried to catch the mink with gloved hand. The reaction of minks was estimated by a score scale ranging from –4 to +5: snorting, false attacks, and bites (aggressive type)—from –1 to –4; cowardice, avoidance of contacts—0; quiet explorative reaction (quiet type)—from +1 to +5 [8]. The females were daily injected intramuscularly in November with chorionic gonadotropin (CG, Profasi-R, Italy) at a dose of 20 IE. The response of the sexual system was estimated by estrous cycle pattern, the ovary function, on the 7th day after the CG injections. There were studied 49 non-selected and 26 females in aggressive and quiescent groups on the 7th day after the CG injections, as well as 8 intact females, accordingly, eight animals in each group.

Stages of the estrous cycle were determined in vaginal smears that were taken with humid cotton tampons on the 7th day after CG administrations, placed on a ruled glass panel, dried, fixed in 96% ethanol (10 min), and stained with a 1% orcein (10 min); the sexual cycle stage was estimated under microscope at a 100-fold magnification. The anestrus stage (A) was identified from the presence of parabasal intermediate cells and leucocytes, the proestrus (P)—from polygonal epithelial cells, estrus (E)—from nucleated and anucleated keratinized epithelial cells. The samples containing a mixed cell population were considered to be typical of transitory stages—anestrus-proestrus (AP) and proestrus-estrus (PE).

In each group of the females, ovaries were removed for histological study. They were cleared from adjacent tissues and fixed in Lilie solution. After corresponding procession they were embedded in paraffin, serial sections were cut and stained with hematoxylin–eosin. On the of 8-mm thick ovary serial sections the number of follicles was counted using the method of Pedersen and Peters [9]: the follicles were divided into nine types depending on the degree of development and the number of granulose cell layers. The following types of follicles were identified: growing (the oocyte was surrounded by well-formed granulose cells—one–four and more granulose cell layers—1C, 2C, 3C, i 4C); maturating (the appearance of follicle cavity—AFC, the well-formed cavity—WFC, Graafian vesicle—GV); atretic follicles A1 (the beginning of follicle atresia without structural rearrangement of cell elements), and A2 (the beginning of rearrangement of follicle cell elements).

After sacrifice of the minks, hypothalamus was removed and placed into liquid nitrogen. Subsequently the brain samples were kept at −80°C until analysis. The DA and NA contents were measured by method of Schlumpf and co-authors [10].

RESULTS

Injections of CG produce ovulation in mink females in 35–40 h [11, 12], which synchronizes development of the estrous cycle. At the 7th day after CG administrations, the selected for behavior and non-selected females differed essentially by