EXPERIMENTAL BIOLOGY

Parameters of Estrous Cycles in Albino Rats Normally and after Injection of Xenogenic Cerebrospinal Fluid

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We studied biological effects of xenogenic cerebrospinal fluid. Injection of the liquor to albino female rats during different periods of postnatal ontogeny induced changes in the dynamics of estrous cycles. Changes in the picture of vaginal smears, duration of estrous cycles, and proportion between their phases were found.

Key Words: *cerebrospinal fluid; estrous cycle; ovary*

The cerebrospinal fluid (CSF), a humoral medium of the CNS, interacts with brain cells and contains a wide spectrum of bioactive substances. It contains hormones of the hypothalamus, pituitary, pineal gland, and peripheral endocrine glands, neuropeptides, neurotransmitters, endogenous opiates, growth factors, and other metabolites essential for the regulation of the reproductive function [5-7]. Parenteral injection of CSF causes no immunopathological reactions due to low protein content in the liquor and specific immune status of the brain separated from the blood by the blood-brain barrier (BBB) [3]. Cattle CSF is most close to human CSF by its composition and is easily available. Various effects of parenteral injections of CSF were demonstrated and prospects of its use as the drug base were outlined [1]. The characteristics of CSF depend on the donor status (health or disease), determining the hormone content in it. Study of CSF characteristics is of theoretical and practical interest for the development of drugs for veterinary and medicine on the base of an available biological substratum.

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We attempted to detect heretofore unknown effects of the CSF on the reproductive system of polyestrous mammals after its injection during different periods of postnatal ontogeny.

MATERIALS AND METHODS

Since lactation is the cause of anovulation, we used CSF of lactating cows (week 2 after delivery), expecting an inhibitory effect of the CSF on the reproductive system of the recipients. CSF (100-150 ml) was collected by suboccipital puncture and was stored in liquid nitrogen.

Wistar rats were divided into 5 groups, 10 per group: 1) newborn rat pups during day 1 of life; 2) young (immature) rats (30 days); 3 and 4) adult (mature) rats (100 days). CSF was injected intraperitoneally (2 ml/kg). Rats of groups 1, 2, and 3 received a single dose of the liquor (0.015, 0.1, and 0.5 ml, respectively). Group 3 rats were injected with CSF during the diestrus phase. Group 4 rats were injected with the CSF every other day during 30 days in a dose of 0.5 ml (8 ml per course). Controls were injected with 0.9% NaCl according to the same protocol.

The experiment was carried out in spring-summer: the rats were born in March, the smears were

analyzed in June-July from day 80 (before the onset of cycles) though days 140-150 of life. The rats were kept under standard vivarium conditions; the pups were taken from litters with equal male/female ratio (8-10 pups) and were separated from mothers at the age of 28 days.

The duration of the estrous cycle, number of days per stage of the cycle, cycle phase coefficients, cytological differences in vaginal smears collected during analogous cycle phases in experimental and control rats were evaluated. Daily analysis of smears collected at fixed hours was carried out in all rats aged 80-120 days. The mean duration of intervals between two proestrus phases over 30 days was considered as the mean duration of the estrous cycle in each female. The cycle phase coefficients were calculated by the formula: $K=a/b\times 100\%$, where a is the duration of the cycle phase (in days) during the period of observation, b total duration of the study in days (30 days in our study).

The proestrus, estrus, metestrus, and diestrus coefficients were calculated. Short proestrus characterized by the presence of nucleated cells and scales in the smear was taken for the sum of $^1/_2$ estrus and $^1/_2$ proestrus. The absolute duration of each phase of the cycle was not determined, but the number of days when it was registered at 12.00 was taken into consideration. Each stage of the cycle takes 1 day in normal rats (according to analysis of the smears collected at 12.00).

The data on the cycles from the moment of their onset were statistically processed for 30 days. The significance of differences was evaluated using Student's t test.

RESULTS

Delayed body weight gain and terms of vagina opening and first estrus were observed in rats of groups 1 and 2, which indicated delayed onset of puberty (Table 1).

The total duration of the estrous cycles in all groups of experimental animals was significantly prolonged ($p<0.01$), but the total rhythm of cycles remained normal: 5.09±0.23 in group 1, 5.22±0.28 in group 2, 4.57±0.12 in group 3, 6.73±0.67 in group 4, and 4.07±0.09 days in the control (Fig. 1).

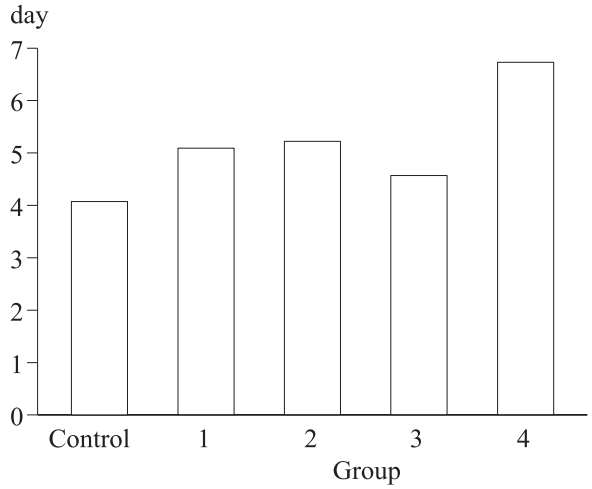

Fig. 1. Duration of estrous cycles in intact and experimental rats.

The estrous cycles were prolonged at the expense of the estrus and diestrus phases, while the proestrus phase was shortened (Table 2). The diestrus phase was prolonged in groups 1 and 2 (CSF injections before puberty), while in group 3 the estrus phase was prolonged. In group 4 the cycles were prolonged at the expense of long estrus.

Analysis of the smears on the next day after CSF injections showed that vaginal epithelial basal layer cells formed extensive accumulations in experimental rats (this picture was not observed in controls). By contrast, the proestrus was prolonged in the majority of subsequent cycles. Despite longer duration of the estrus in group 4 rats, the microscopic picture of the smears was identical to that in controls. A significant increase in the number of leukocytes in smears collected during the metestrus and diestrus phases was observed in experimental animals.

Abundant transparent viscous mucus was detected in vaginal smears from experimental rats of groups 1 and 2 at the age of 100 days; together with the absence of cyclic changes this reflected

TABLE 1. Parameters of Puberty in Rats of Groups 1 and 2 ($M\pm m$)

Group		Day of the vagina opening	Day of the first estrus	Body weight at the age of 90 days, g
1	control	53.6±0.6	87.5±1.2	161.0±1.9
	experiment	83.9±0.7*	116.6±1.1*	104.4±2.7*
2	control	53.3±0.6	84.8±0.8	167.6±2.6
	experiment	64.4±0.6*	102.4±1.6*	165.3±1.9

Note. *$p<0.001$ compared to respective control.

TABLE 2. Coefficients of Estrous Phases in Experimental and Control Rats (%; *M*±*m*)

Group	Coefficient of				
	short proestrus	proestrus	estrus	metestrus	diestrus
Control	1.67±0.59	23.83±0.64	25.50±0.64	25.67±0.75	25.00±0.59
1	9.33±1.46***	13.67±1.07***	27.33±1.53	25.00±1.41	34.00±3.05*
2	9.33±1.95**	14.33±1.15***	26.33±1.67	26.00±1.95	33.33±2.72*
3	8.33±1.41**	18.50±1.45**	31.17±0.91***	28.00±1.30	22.33±0.92*
4	8.67±1.50**	7.67±1.66***	45.00±6.40**	22.00±2.88	25.33±4.95

Note. *p<0.05, **p<0.01, ***p<0.001 compared to the control.

delayed puberty. No smears of this kind were detected in control rats aged 90 days. With growth and maturation of experimental animals, vaginal epitheliocytes appeared in the smears, gradually becoming more numerous than leukocytes; the volume of the mucus decreased, and the estrous cycle was established within 4-10 days.

Changes in the estrous cycles were permanent over the period of observation (2 months since the onset of cycling) in groups 1 and 2, reversible in group 3 (the parameters normalized within 24-30 days), and irreversible over the period of 2 months since the start of CSF injections in group 4.

The first 24 h of life are referred to the perinatal period characterized by intensive processes of sexual differentiation of the neuroendocrine system, first meiotic division of oocytes, and formation of primordial follicles. This age is characterized by functional immaturity and high permeability of BBB [2,4]. Cavitary follicles appear in rat ovaries at the age of 30 days (early stage of puberty). The estrous cycle established in rats at the age of 90 days. The described changes in the estrous cycles during treatment with CSF indicate restructuring of the neurohumoral and reproductive systems. Many authors noted the possibility of modification of the neurochemical systems of the brain under the effects of various factors during critical periods of ontogeny [2,4]. We detected diverse effects of CSF injections before and after the onset of puberty. Presumably, this is explained by the fact that the CSF effect is realized by the central mechanism during the perinatal period, because of immature BBB structures, while in adult animals it is realized by the peripheral mechanism. The main organ regulating the estrous cycles is the ovary [2], the sequence of events in one cycle being an obligatory condition for the formation of the next estrous cycle, which explains the lasting effect of CSF injections.

Hence, injections of xenogenic CSF to female rats delayed the onset of puberty and prolonged estrous cycles at the expense of the estrus and diestrus phases. Changes in the cycles in animals induced by injections of CSF before puberty were opposite to those in adult females. Repeated injections of the CSF cause similar, but more pronounced changes in the estrous cycles.

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