Neuroscience Letters 495 (2011) 192–195

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Acceleration of irregular estrous cycle in forced running by midbrain raphe lesions in female rats

Hideo Shimizu [a], Korehito Yamanouchi [a,b,*]

[a] Neuroendocrinology, Department of Human Behavior and Environment Sciences, Faculty of Human Sciences, Waseda University, 2-579-15, Mikajima, Tokorozawa, Saitama 359-1192, Japan
[b] Advanced Research Center of Human Sciences, Waseda University, 2-579-15, Mikajima, Tokorozawa, Saitama 359-1192, Japan

ARTICLE INFO

Article history:
Received 15 November 2010
Received in revised form 18 March 2011
Accepted 22 March 2011

Keywords:
Raphe nucleus lesion
Estrous cycle
Forced running
Stress
Female rats

ABSTRACT

It is known that over-exercise or forced running interrupts the regular ovulatory (estrous) cycle in female mammals, including women. The serotonin content of the brain changes under stress conditions. In this experiment, radiofrequency lesions were made in the dorsal (DRL) or median (MRL) raphe nuclei of the midbrain, in which serotonergic neurons are abundant, and changes in the estrous cycle with forced running using an electric-motor running wheel were examined in female rats. Through the tests, the estrous cycle was checked by taking vaginal smears. Female rats with a regular 4-day estrous cycle were forced to run in the wheel for 30 min daily over 15 days. As a result, 27.3% of the control and 30.0% of the sham-operated rats showed an irregular estrous cycle. In contrast, 100% of the DRL and 87.5% of the MRL rats showed an irregular cycle ($P < 0.05$ vs. control and sham). Statistical analysis revealed that the median onset day of an irregular cycle was in excess of 15 days in both the control and sham groups. In the DRL and MRL groups, the median onset days of the irregular cycle were day 5 and 3, respectively, being shorter than those in control and sham groups ($P < 0.01$). These results indicate that the dorsal and median raphe nuclei play an important role in preventing the effect of stress conditions in the ovulatory system in female rats.

Healthy adult female rats show a regular 4-day ovulatory cycle; proestrus, estrus, diestrus 1 (metaestrus) and diestrus 2. The ovulatory cycle is regulated by the hypothalamo-pituitary-ovarian axis [see review [8]]. Increase in estrogen secreted from a matured follicle in the morning of the day before ovulation is thought to act on the preoptic area (POA) and cause a surge in gonadotropic hormone-releasing hormone (GnRH) followed by a surge in luteinizing hormone (LH), which induces ovulation. Other than the ovulatory cycle, the change in the blood level of estrogen causes the 4-day cycle of uterine and vaginal conditions. Because of the regular 4-day change in the epithelial cells of the vagina corresponding to the change in the estrogen level, the ovulation cycle can be identified easily by checking a vaginal smear under the microscope.

The ovulatory cycle and/or follicular development are interrupted by external stresses, such as constant light [6] and high temperature [26], that induce disruption of the hormonal and neural conditions. A similar anovulatory condition is known to be induced by internal stress. In human females, over-exercise [36] or mental exhaustion [7] can cause dysfunction of the reproductive functions such as anovulation. In rats, ovulation can be blocked by forced immobilization [35]. It has been reported that chronic cold stress induced disruption of GnRH-mRNA expression in female rats [31].

Much evidence has shown that the serotonergic neural system is involved in stress conditions. Forced swimming and other stress increased or decreased amounts of extracellular serotonin and metabolites with regional differences in the male rat brain [15,16]. On the other hand, physical or psychological stress caused an increase in the serotonin content with differences dependent on the intensity of stress in the forebrain in male rats [11].

Furthermore, the hypothalamo-pituitary-adrenal axis, which is influenced by stress conditions, affects the serotonergic system in the brain. Chronic stress increases plasma corticosterone [1] and corticotrophin-releasing factor (CRF) [9]. Expression of CRF receptor-mRNA in the dorsal raphe nucleus was seen [3,29]. Direct application of CRF to the dorsal raphe has been reported to inhibit the activity of serotonergic neurons [30]. Stress condition or glucocorticoids increases the expression of serotonin receptors 1A and decreases 2A receptors [18] in male rats.

Most serotonergic neurons in the brain exist in the raphe nuclei of the lower brainstem [33]. The dorsal (DR) and median (MR)

* Corresponding author at: Laboratory of Neuroendocrinology, Department of Human Behavior and Environment Sciences, Faculty of Human Sciences, Waseda University, 2-579-15 Mikajima, Tokorozawa, Saitama 359-1192, Japan.
Tel.: +81 4 2947 6727; fax: +81 4 2947 6727.
E-mail address: hedgehog@waseda.jp (K. Yamanouchi).
URL: http://www.f.waseda.jp/hedgehog/ (K. Yamanouchi).

0304-3940/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2011.03.063

raphe nuclei in the midbrain contain half of the serotonergic neurons in the brain and send serotonergic axons to the forebrain [2]. The hypothalamic nuclei, such as the preoptic and ventromedial hypothalamic areas (VMH) that are responsible for the regulation of ovulation, receive serotonergic axons from the DR and MR [13]. Destruction of the DR decreases serotonin and its metabolites in these areas in male rats [12]. Serotonin is one of the important factors in the neural control of ovulation and the ovulatory cycle, because deprivation of serotonin by treatment with a synthesis inhibitor was found to block a rise in LH [4]. Lesion of the DR interrupted estrous cyclicity and the preovulatory LH surge in female rats [25,34]. Furthermore, the DR plays an important role in the ovulation-triggering mechanism in the forebrain through the serotonin 2A/2C receptor [21].

However, the role of the raphe nuclei in the mechanism underlying anovulatory syndrome caused by stress conditions has not yet been analyzed. In the present study, as one step to elucidate the mechanism, the DR or MR was lesioned and the ovulatory cycle was checked under the condition of over-exercise by forced running using an electric drive running wheel in female rats.

Adult female Wistar rats (300–380 g, Takasugi Animal Farm, Saitama, Japan) were kept under a controlled temperature (23–25 °C) and photoperiod (LD 14:10, light off at 19:00 h). Food and water were accessed ad libitum. A vaginal smear was checked every morning through the experiment and rats that had two or more consecutive regular 4-day estrous cycles were used in this experiment. Female rats underwent radiofrequency lesioning of the midbrain raphe nuclei. After surgery, the rats were housed two to a cage. Animals ran forcedly for 30 min every day in a 19-day period using an electric running wheel and the animals were checked to determine the condition of their vaginal estrous cycle. All experiments were conducted according to the regulations for Animal Experimentation at Waseda University (Permission No. 09J005).

The DR or MR was lesioned on the day of estrus. Under isofluran anesthesia, the rats were fixed in the stereotaxic instrument in which the incisor bar was set at 3.3 mm below the interaural line. In the dorsal raphe nucleus lesion group (DRL, 8 rats), an electrode with a diameter of 0.7 mm was lowered 6.5 mm from the bregma level at a point 7.8 mm posterior from the bregma on the midline. In the median raphe nucleus lesion group (MRL, 8 rats), the electrode was lowered 8.5 mm from the bregma level at the same point of the DRL. In both lesion groups, current was applied and the temperature of the tip of the electrode was kept at 57 °C for 1 min (RGF-4A, Radionics Inc., Burlington, MA). In the sham group (10 rats), the electrode was lowered at the same point of the MRL but current was not applied. As the control group, 11 intact rats were prepared.

After rats with DRL or MRL showed 2 or more regular estrous cycles, forced running was started on the day when the rats exhibited vaginal estrus. At 15:00–16:00 h, all animals were put into the electric running wheel (self-turning wheel cage) and forced to run for 30 min everyday for 19 days. The diameter and width of the electric wheel was 35 cm and 10 cm, respectively. During the first 4 days (day 1–4) of adaptation to the wheel, the running speed was 5 rpm. From day 5 to 19 (15 days), the speed of the wheel was 15 rpm.

When the rat showed two consecutive disappearances of vaginal proestrus, the animals were regarded as anovulatory (acyclic state). The first day of disappearance of vaginal proestrus was determined to be the day of onset of the acyclic condition in this experiment.

After the forced running, animals were autopsied by over-dose of ether. The brain was removed and fixed in 10% formalin solution to determine the precise localization of the lesion. Next, frozen sections, 100 μm in thickness, were made and stained with cresyl fast violet.

Comparison of the incidence of the irregular estrous cycle among the groups was analyzed using the chi-square test. The difference in the median onset day of disappearance of the estrous cycle was analyzed by the Mann–Whitney *U*-test. For all statistical tests, differences were considered to be significant at $P < 0.05$.

Table 1
Incidence of irregular estrous cycle by forced running in female rats with dorsal (DRL) and median (MRL) raphe nucleus lesions.

Groups	No. of rats	Irregular estrous cycle		Body weights
		Incidnece	%	
Control	11	3/11	27.3	396.4 ± 48.0
Sham	10	3/10	30.0	369.0 ± 60.1
DRL	8	8/8*	100.0	376.3 ± 58.3
MRL	8	7/8**	87.5	337.5 ± 43.3

* $P < 0.01$ vs. Control and $P < 0.02$ vs. Sham.
** $P < 0.05$ vs. Control and Sham.

As the results, after end of the forced running, body weights in DRL and MRL groups were almost same as those of the control and sham groups (Table 1). After receiving lesions, locomotor and behavioral activities of DRL and MRL rats in the home cage in the light phase were not different from those in control groups, although systematic analyses has not been done in this experiment.

Check of vaginal smear showed after destruction of the DR, next P phase did not appear in 3 of 8 rats and diestrous condition continued until 6.7 ± 0.3 days after surgery in these animals but 5 remains showed regular 4-days cycle. On the other hand, in all of the MRL rats, regular estrous cycle was not disturbed by MRL.

During forced running tests, vaginal smear check showed P phase disappeared 3, 7 or 11 days after the start and diestrus was continued until end of the test in 3 of 11 control rats and remains showed regular cycle during the observation periods (Table 1). In the sham group, P phase disappeared 3 or 7 days in 3 of 10 rats and remains showed regular estrous cycle. In contrast, all of 8 DRL became irregular estrous cycle by forced running. In 4, 3 or 1 DRL rats, P phase disappeared 3, 7 or 11 days after start, respectively and after disappearance of P phase, diestrous condition was continued until the end of the test (Fig. 1). Similar results were obtained in the MRL group, 7 of 8 rats became an irregular cycle. P phase disappeared 3 or 7 days after start in 6 or 1 MRL rats, respectively and after disappearance of P phase, diestrous was continued.

The incidences of irregular estrous cycle in the DRL and MRL groups were statistically higher than those in the control and sham groups ($P < 0.05$).

The median day of disappearance of vaginal proestrus (onset day of the irregular estrous cycle) in each group is shown in Fig. 2. Statistical analysis revealed that the onset day of the irregular cycle in both the control and sham groups was in excess of 15 days (control; min = 3 and max = 15<, sham; min = 3 and max = 15<). In the DRL and MRL groups, onset of the irregular cycle was day 5 (min = 3 and max = 11) and day 3 (min = 3 and max = 15<), respectively, which was shorter than in control and sham groups ($P < 0.01$).

The location of the raphe lesions was determined histologically following the brain atlas of Paxinos and Watson [27] (Fig. 3). In the DRL group, lesions were seen at the level from the oculomotor nucleus to the locus coeruleus on the midline of the midbrain. The main body of the DR and adjacent areas in the central gray of the midbrain was damaged. The DRL penetrated the decussation of the superior cerebellar peduncle in some rats. In the MRL group, lesions were seen in the antero-posterior axis at the same level of the DRL. The main body of the MR and adjacent areas in the tegmentum were damaged. In the MRL group, lesions also extended to the decussation of the superior peduncle in some rats.

In this experiment, DRL and MRL accelerated the onset of an irregular estrous cycle as a result of forced running in female rats. This indicates that midbrain raphe nuclei play an important role in protecting the regular ovulatory cycle from the effects of stress