Roles of catecholamine at the preoptic region in the regulation of cyclic ovulation and gonadotropin

Ryuhei Hashimoto and Fukuko Kimura

2nd Department of Physiology, Yokohama City University School of Medicine, Yokohama, Japan

Abstract. Experiments were designed to see how a transplantation of newborn norepinephrine (NE) neurons from A-6 groups or dopamine (DA) neurons from A-10 groups in the third ventricle at the level of the preoptic region affected the vaginal oestrous cycle and gonadotropin secretion in the female rat. Sixty-two rats that had tissues in contact with the preoptic region were evaluated as having surviving transplants by histological examination after sacrifice. The rats that had surviving NE-neuron transplants frequently showed prolongation of oestrus during the 70-day study period, indicating that ovulation was severely impaired in these rats. However, after ovariectomy, they showed a pulsatile secretion of LH with a remarkably large amplitude. The DA-neuron transplants sustained the oestrous cycle unchanged, but increased blood levels of FSH prior to the ovulatory secretion of gonadotropin. Pulsatile LH secretion was not affected. Sham and cerebellum control rats did not show any significant changes in the oestrous cycle. The results suggest that the NE-neuron transplants at the preoptic region somehow inhibit gonadotropin secretion in intact rats, whereas they facilitate it in ovariectomized rats. The DA-neuron transplants appear to exert facilitatory effects on FSH secretion in intact rats.

It is well established that brain catecholamines (CA) play a significant role in the regulation of gonadotropin secretion (for review, see Ramirez et al. 1984). Further, there is increasing evidence that a periventricular suprachiasmatic structure of the preoptic region plays a key role in the regulation of cyclic gonadotropin secretion (Kawakami et al. 1977; Wiegen & Tarasawa 1982). In the present study, we examined the role of norepinephrine (NE) and dopamine (DA) in this preoptic region by a brain transplantation method, which has been introduced and extended by Björklund et al. (1980). It was expected that certain changes might occur in the reproductive function if CA neurons were transplanted into the preoptic region of a cycling rat.

Materials and Methods

Adult female Wistar rats as recipients and pregnant rats of the same strain to obtain newborn rats to use as the tissue donors were purchased, and maintained under conditions of controlled temperature (24–26°C) and illumination (lights on 05.00–19.00 h).

The tissue, including A-6 group NE neurons in the locus coeruleus (for NE-rich transplant) or A-10 group DA neurons in the substantia nigra (for DA-rich transplant) was punched out of the coronal slice dissected from the medulla or midbrain of newborn rats, respectively, with a metal cannula and was stereotaxically positioned in the third ventricle at the level of the preoptic region of a recipient rat anaesthetized with pentobarbital sodium. Tissues from the cerebellum were transplanted as control transplants, and an empty cannula was inserted and pulled out for sham controls.

Vaginal smears were obtained every morning for about 10 weeks post-operatively. After observations of the vaginal oestrous cycle were finished, some rats were ovariectomized to serve in the experiment on pulsatile LH secretion. All rats were sacrificed at the end of study by decapitation between 12.00 and 14.00 h. Trunk blood was collected for hormone measurement and
ovarian and uterine weights were recorded for each animal. The brains were removed, fixed in 10% formalin, sectioned serially, and stained with cresyl violet, the Klüver-Barrera stain. Approximately half the number of brains were subjected to CA fluorescence examination (Fall et al. 1962; Corrodi & Jonsson 1967). LH and FSH concentrations in serum were measured by the double-antibody radiimmunoassay using kits supplied by the NIH-LH-Sl and NIH-FSH-Sl.

Results

1. Histological examinations of the transplants (Table 1)
A total of 95 female rats received intraventricular transplantations of neonatal CNS tissues, but only 62 were evaluated as having surviving transplantsin contact with the preoptic region after histological examination. The remaining 33 rats did not have transplants detectable or had only necrotic tissues in the third ventricle. Surviving transplants had normal Klüver-Barrera stained neurons and were found to contact with the ependymal layer of the recipient brain which demonstrated a high degree of vascularization. The brains which were subjected to CA fluorescence method provided evidence that the tissues from the locus coeruleus and substantia nigra contained CA neurons.

2. Oestrous cycle (Figs. 1 and 2, Table 1)
When the dioestrus and oestrus lasted for periods of more than 4 and 3 days, respectively, they were arbitrarily called prolonged dioestrous and oestrus, respectively. Prolonged dioestrous occurred in the majority of sham control rats either within 2 weeks or afterwards, indicating non-specific effects exerted by surgical operation and experimental manipulation. Prolonged oestrus never occurred. The changes in vaginal smears in rats that received cerebellar tissue transplantations were, irrespective of their survival, almost exactly the same as those in sham control rats. Similarly, although several rats with surviving DA-rich transplants had prolonged dioestrous or oestrus, statistical analysis revealed no significant difference between the rates in sham control rats and rats with surviving cerebellar tissue.

Twenty-nine of 43 rats with surviving NE-rich transplants showed prolonged dioestrous either within the initial 2 weeks or afterwards, but there were no significant differences when comparing the rates in sham control rats and in rats with

<table>
<thead>
<tr>
<th>Transplants</th>
<th>No. of rats</th>
<th>Surviving transplants</th>
<th>No. of rats (%)</th>
<th>No. of rats that showed</th>
<th>Prolonged dioestrous</th>
<th>Prolonged oestrous</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Initial 2 weeks</td>
<td>Overall</td>
<td>Initial 2 weeks</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham control</td>
<td>11</td>
<td>11</td>
<td>8 (73%)</td>
<td>10 (91)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Cerebellum tissue</td>
<td>6</td>
<td>+ 4 (67)</td>
<td>3 (75)</td>
<td>4 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- 2</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>DA-rich tissue</td>
<td>23</td>
<td>+ 15 (65)</td>
<td>11 (73)</td>
<td>14 (93)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- 8</td>
<td>4 (50)</td>
<td>6 (75)</td>
<td>0 (0)</td>
<td>2 (25)</td>
</tr>
<tr>
<td>NE-rich tissue</td>
<td>66</td>
<td>+ 43 (65)</td>
<td>23 (53)</td>
<td>29 (67)</td>
<td>16 (37)</td>
<td>41 (95)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- 23</td>
<td>13 (57)</td>
<td>14 (74)</td>
<td>2 (9)</td>
<td>5 (22)</td>
</tr>
</tbody>
</table>

The effects of CNS tissue transplants on the cyclic ovulation were estimated by analysing prolonged dioestrous and oestrus; the numbers of animals that showed prolonged dioestrous and oestrus were calculated for the period examined and also for the period of 2 weeks immediately after the operation. a: $P < 0.05$ vs sham control, b: $P < 0.005$ vs surviving cerebellum tissue, and c: $P < 0.005$ vs not-surviving tissue, as analyzed by the chi-square test.
Changes in vaginal oestrous cycles in rats that received sham operation (upper, dashed lines), cerebellum tissue transplantation (upper, solid and dotted lines) and dopamine-rich tissue transplantation (lower, solid and dotted lines). In this and the next figure, only prolonged dioestrus and oestrus are exaggerated for simplicity; when the dioestrous and oestrous states lasted for periods of more than 4 and 3 days, respectively, (shown with open and solid bars) based on the fact that in the ordinary oestrous cycle, dioestrus and oestrus last for 2 or 3 days and 1 or 2 days, respectively. Further, for the rats with CNS transplantation, oestrous cycles in rats with surviving transplants are shown with solid lines and those in rats with not-surviving ones are shown with dotted lines.

3. Hormone concentrations in blood and organ weights (Fig. 3)
In DA-rich tissue transplanted rats, LH levels were not different in either dioestrus or pro-

surviving cerebellar transplants. The most striking change seen in vaginal smears of rats with surviving NE-rich transplants was the occurrence of prolonged oestrus with repetitive and long-lasting features. Prolonged oestrus appeared even immediately after the operation for NE-rich tissue transplantation in 37% of rats, suggesting pharmacological effects of NE that may have leaked from the transplants. When comparing rats with and without surviving NE-rich transplants, the occurrence of prolonged oestrus was significantly higher in rats with surviving tissue, whereas there was no difference in the rate of occurrence of prolonged dioestrus. Histological examination of ovaries of rats that had shown prolonged oestrus demonstrated that they contained a considerable number of follicles without fresh corpora lutea.

Fig. 1.
Changes in vaginal oestrous cycles in rats that received norepinephrine-rich tissue transplantation. Further details, see Fig. 1.

oestrus from those in sham and cerebellum control rats, whereas FSH levels were higher than in the controls; the difference was significant in pro-oestrus, although not in dioestrus. There were no significant differences in the LH level in rats with NE-rich transplant in dioestrus and pro-oestrus. However, in rats in prolonged oestrus, the LH level was significantly higher than in rats in pro-oestrus with NE-rich transplants.

Ovarian weight was not different between groups. Uterine weight was greater in rats in dioestrus with DA-rich transplants, but was lower
in rats in pro-oestrus with NE-rich transplants. No difference was found for rats in prolonged oestrus. The weight of intrauterine fluid was increased in rats with DA-rich transplants, whereas it was quite low in rats with NE-rich transplants.

4. Pulsatile LH secretion after ovariectomy
Pulsatile fluctuations in serum LH levels were evident at glance in all rats tested. When the characteristics pulses were analysed as described previously (Akema et al. 1984), it was found that the rats transplanted with NE-rich tissue exhibited a significantly ($P < 0.01$) larger LH pulse amplitude: $10.06 \pm 0.87$ (mean $\pm$ SEM, $N = 5$) vs $6.18 \pm 0.49$ in sham controls ($N = 4$), and overall mean LH concentration: $10.31 \pm 0.61$ vs $8.10 \pm 0.40$ in sham controls. The pulse frequency was not different between groups. There were no significant differences in the pulsatile LH secretion of rats with DA-rich tissue transplant and sham controls.

Discussion
It was extremely interesting that the NE-rich transplant fusing to the preoptic region produced significant alterations in the secretion of gonadotropin in the female rat. First of all, the rats with NE-rich transplants very frequently entered into a reproductive status characterized by a recurrent anovulatory prolonged oestrus. Together with polyfollicular ovaries, smaller uteri and less uterine fluid in these rats, NE-rich transplants appeared to inhibit gonadotropin secretion in intact rats. Second, such animals that had shown prolonged oestrus exhibited, after ovariectomy, a pulsatile secretion of LH with a remarkably large amplitude, indicating that NE-rich transplants facilitated gonadotropin secretion in ovariectomized rats.

The inhibitory effect of NE-rich transplants presumed for intact rats is, however, puzzling in light of well established concept that NE transmission plays a facilitatory role in the CNS control mechanism of LH secretion (see for review of Ramirez et al. 1984). However, a possible explanation is that NE released from transplants exerts an inhibitory effect through a $\beta$-receptor instead of an $\alpha$-receptor mechanism. The $\beta$-receptors are considered to mediate the action of inhibitory component of ascending NE bundles, both in intact (Caceres & Taleisnik 1982) and in ovariectomized rats (Leung et al. 1981), and such NE fibres have terminals in the preoptic region (Day et al. 1980). Even so, there arises a question why in ovariectomized rats the activation of $\beta$-receptor does not lead to the suppression of pulsatile LH
secretion. This is probably related to the number of these receptors that changes depending on the steroid treatment, but the precise mechanism remains to be studied. Alternatively, it could be due to the densitization that would have occurred in the LHRH secretory system as the result of prolonged stimulation by NE. It has been shown that the GnRH secretory system rapidly develops desensitization in response to NE infused continuously in the third ventricle of ovariectomized oestrogen-primed rats (Gallo 1982). However, this is less reliable considering the facilitation of LH secretion in ovariectomized rats, since administration of a-agonists in the third ventricle or medial preoptic region inhibited pulsatile LH secretion acutely in ovariectomized rats (Leung et al. 1982).

There was no significant change in the oestrous cycle of rats with the DA-rich transplants, but serum FSH levels were higher than in controls, without changes in LH levels. The increase in FSH secretion was assumed to be followed by an increase in ovarian oestrogen secretion, considering the greater weights of the uterus and intrauterine fluid in those rats. We found previously that the implantation of DA in the preoptic region induced an ovulatory surge-like LH secretion in ovariectomized oestrogen-primed rats (Kawakami et al. 1979). Significant implication of DA transmission at the level of the preoptic region in the control of gonadotropin secretion is supported by a recent histological finding that several-fold more cell bodies and fibres are present in the preoptic periventricular region of the female than in male rats (Simerly et al. 1985). Although elevated FSH secretion, followed by elevated oestrogen secretion, seemed incapable of inducing an advanced LH surge in the present study, more detailed experiments will be of value to identify the role of DA in the control of ovulatory gonadotropin secretion.

Although we have emphasized the locus coeruleus and substantia nigra as neural substrates which contain abundant CA neurons, it is also possible that neurons of these areas produce some other chemical substances, such as peptides, which would exert an action as that observed in the present study. Further, it is also plausible that an action of CA or some other substance takes place at a site other than the preoptic region; it may exert an action via the CSF somewhere else in the brain.

Acknowledgment

This study was supported by a Grant-in-Aid for Scientific Research (No. 59480114) from the Ministry of Education, Science and Culture of Japan.

References


Received February 18th, 1987.
Accepted July 6th, 1987.

Dr F. Kimura,
Department of Physiology,
Yokohama City University School of Medicine,
3-9 Fuku-ura, Kanazawa-ku,
Yokohama 236,
Japan.