An Autoradiographic Study on the Incorporation of $^3$H-leucine in the Brain of Ovariectomized Rats after Administration of Sex Steroids

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Synopsis

A single subcutaneous injection of progesterone, estradiol-17β or oil was given to castrated rats. One and half hr after the injection, a single injection of $^3$H-leucine was given intraperitoneally to animals. The incorporation of the radioactive material in the brain was studied autoradiographically 2 hr after the injection of $^3$H-leucine. The radioactivity in various brain areas was measured by counting the number of reduced grains over cell bodies of neurons.

In the castrated controls, high incorporation of $^3$H-leucine was observed in the supraoptic and paraventricular nuclear regions and Ammon's horn. Treatment with either progesterone or estradiol-17β induced a significant enhancement of the incorporation of radioisotope in the arcuate, ventromedial and supraoptic nuclei, and Ammon's horn. Not estradiol-17β but progesterone caused an increase in the incorporation of $^3$H-leucine in the suprachiasmatic and anterior periventricular nuclear regions, whereas the incorporation of the isotope in the paraventricular nucleus and the nucleus of mammillary body was enhanced only by estradiol-17β.

Possible reasons for high incorporation of the radioisotope in the supraoptic and paraventricular nuclei, the arcuate and ventromedial nuclei and the suprachiasmatic and anterior periventricular nuclei in hormone-treated animals were discussed.

Substantial evidences have accumulated, suggesting that $^3$H-estradiol is selectively taken up from the blood and retains in the hypothalamus and anterior hypophysis (Kato and Villee, 1967 a; Kato and Villee, 1967 b; Eisenfeld and Axelrod, 1965; McGuire and Lisk, 1968; Pfaff, 1968 a; Pfaff, 1968 b) and that the hormone seems to affect the pituitary function and mating behavior in many species, including rats (Harris and Cambell, 1966; Donovan, 1966; Sawyer, 1960). On the other hand, although progesterone also seems to be an important factor in regulation of the pituitary function, feedback action on the hypothalamus and mating behavior, the binding of this hormone to the hypothalamic nuclei has not been demonstrated in the short-term experiments (Laumas and Farooq, 1966; Seiki et al., 1968; Seiki et al., 1969). Moreover, Ross and Clemens (1969) have suggested that progesterone may act in the midbrain to facilitate sex behavior, hypothalamic uptake of progesterone being unimportant for the measure of the behavior. The present authors (1971) have, however, recently obtained data that in the long-term experiment progesterone may selectively be taken up by the hypothalamus and pituitary gland in female ovariecetomized rats. This provides an evidence for the presence of specific progesterone-binding receptors in the hypothalamus and pituitary gland.

Some brain nuclei have been found to be related to sexual function. Particularly, the
arcuate-ventromedial nuclei in the hypothalamus were regarded as a center which regulates the pituitary-gonadal system (Flerkó, 1963; Barracough, 1967) through the secretion of gonadotrophin releasing factors (McCann and Dhariwal, 1966).

The present experiment was carried out by means of autoradiographic technique to investigate an acute effect of estradiol-17β and progesterone on the incorporation of ³H-leucine in the cell bodies of neurons in various areas of the brain of castrated rats.

**Materials and Methods**

Female rats of Wistar strain were divided into 3 groups; castrated, progesterone-treated castrated and estradiol-17β-treated castrated. Each group consisted of 4 animals. All animals which demonstrated 4 day regular cyclicity and weighed about 240 g were ovariectomized on the day of diestrus. At thirty days following surgery a single subcutaneous injection of sesame oil, 1 mg of progesterone in sesame oil or 100 µg of estradiol-17β in sesame oil was given to respective groups of animals. One and half hr after the administration, ³H-leucine dissolved in distilled water containing 2% ethanol (sp. act. 23 Ci/mM, The Radiochemical Centre, Amersham) was injected intraperitoneally at the dose level of 5 µC/g body weight to each group of animals. Two hr after the injection all animals were killed by cardiac perfusion with 10% neutral formalin under physiological pressure. The brain was removed, embedded in paraffin, and 5 µ serial sections were made.

All the sections were thoroughly washed several times with double distilled water and coated with highly sensitive emulsion (Sakura NR-El, Konishiroku Photo. Ind. Co., Ltd., Tokyo) and then they were maintained in light-proof boxes containing drying agent at 4°C for 7 days. Using the dipping technique in this experiment, 3 µ thickness of emulsion membrane was obtained. After developing all the autoradiographs at the same time, the sections were stained with 1% cresyl violet. The localization of brain areas was confirmed according to the cytoarchitectonic atlas of the rat brain (Szentagothai et al., 1968 a). The numbers of reduced grains over cell bodies were counted in various regions of the brain, including the hypothalamus; anterior periventricular nucleus, suprachiasmatic nucleus, retrochiasmatic region of supraoptic nucleus, the middle part of paraventricular nucleus, the middle part of arcuate nucleus, pars anterior and pars medialis of ventromedial nucleus, pars medialis of mammillary nucleus and Ammon’s horn. For the quantitative evaluation of the autoradiogram, the silver grain number per unit cell area was determined and comparison was made in a similar manner as the previous author (Stumpf, 1968 a). All the silver grain counts were corrected for non-specific background silver grains. Nerve cells of the same size were chosen for the comparison of silver grain number per unit neuron. The silver grains were counted from 100 neurons in 30 sections for each brain area from 4 rats in each group. The average grain number in each brain region was not statistically different in each animal in the same group. The average number of grains per unit cell in various regions of the brain was, therefore, calculated by putting together each grain number per unit cell in the brain regions in the same group.

**Results**

The average numbers of reduced grains over cell bodies in various regions of the brains of 4 castrated, 4 progesterone-treated castrated and 4 estradiol-17β-treated castrated animals are shown in Table 1. In the castrated controls, the cell bodies of neurons of the supraoptic and paraventricular nuclei and the Ammon’s horn showed a fairly high uptake of the radioactive material (Figs. 2–4), compared with that in the remaining cell groups of the hypothalamus (Fig. 1). A single injection of either progesterone or estradiol-17β induced a significant enhancement of the incorporation of ³H-leucine in cell bodies of neurons in the arcuate nucleus (Fig. 5), the ventromedial nucleus (Fig. 6), the Ammon’s horn (Fig. 7) and the supraoptic nucleus (Fig. 8). A single injection of estradiol-17β caused a marked increase of incorporation of

Figs. 1–4. Autoradiographs of the cell bodies of neurons in the castrated rats 2 hr after injection of ³H-leucine. 1. Ventromedial nucleus. 2. Paraventricular nucleus. 3. Ammon’s horn. 4. Supraoptic nucleus. Note the relatively high incorporation of the radiochemical in the supraoptic and paraventricular nuclei. Cresyl violet stain. ×2,000 for 1–3. ×1,500 for 4.
Table 1. Distribution of reduced grains over cell bodies in various regions of the rat brain 2 hr after injection of $^3$H-leucine. Amount of uptake is expressed in the average number of reduced grains per cell with standard error of the mean.

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Castrated (4)*</th>
<th>Castrated and progesterone (4)</th>
<th>Castrated and estradiol-$17\beta$ (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior periventricular nucleus</td>
<td>3.0 ± 0.17</td>
<td>4.7 ± 0.09**</td>
<td>3.0 ± 0.22</td>
</tr>
<tr>
<td>Suprachiasmatic nucleus</td>
<td>3.0 ± 0.16</td>
<td>4.1 ± 0.17**</td>
<td>2.9 ± 0.16</td>
</tr>
<tr>
<td>Supraoptic nucleus</td>
<td>11.9 ± 0.15</td>
<td>19.7 ± 0.48**</td>
<td>16.6 ± 0.55**</td>
</tr>
<tr>
<td>Paraventricular nucleus</td>
<td>14.4 ± 0.71</td>
<td>14.5 ± 0.90</td>
<td>21.5 ± 0.90</td>
</tr>
<tr>
<td>Arcuate nucleus</td>
<td>4.0 ± 0.20</td>
<td>5.8 ± 0.31**</td>
<td>6.5 ± 0.21**</td>
</tr>
<tr>
<td>Ventromedial nucleus</td>
<td>6.7 ± 0.28</td>
<td>8.9 ± 0.41**</td>
<td>11.0 ± 0.38**</td>
</tr>
<tr>
<td>Mammillary nucleus</td>
<td>3.9 ± 0.20</td>
<td>4.0 ± 0.21</td>
<td>5.9 ± 0.36**</td>
</tr>
<tr>
<td>Ammon’s horn</td>
<td>12.9 ± 0.50</td>
<td>15.8 ± 0.50**</td>
<td>17.1 ± 0.70**</td>
</tr>
</tbody>
</table>

* Number of animals in parentheses.
** P < 0.005, compared with the respective control values of the first column.

Discussion

The choice of 1 and a half hr as the interval between hormone treatment and $^3$H-leucine injection, followed by 2 hr as the interval between injection of the radioisotope and decapitation of animals, was dependent upon our finding (Seiki and Hattori, 1971) that the hypothalamus of an ovariectomized rat showed the specifically high uptake of radioactive progesterone during these time intervals and the report (Kato and Villee, 1967b) that the various parts of the hypothalamus showed the relatively high radioactivity for 1 to 4 hr after the injection of tritiated estradiol, as compared with that in the cerebral cortex. An important question of whether the silver grains over cell bodies of neurons in various brain areas came from the incorporated $^3$H-leucine in cell elements and/or from the free $^3$H-leucine would be excluded by the fact that in this experiment all the tissue sections were carefully thoroughly washed several times with double distilled water.

In the castrated rats, the higher incorporation of $^3$H-leucine was observed in the cell bodies of neurons in the supraoptic nucleus (Fig. 9) and the mammillary nucleus (Fig. 10), whereas progesterone did not. On the contrary, a single injection of progesterone induced a slightly high incorporation of the radioisotope in cell bodies of neurons in the anterior periventricular nucleus (Fig. 11) and the suprachiasmatic nucleus (Fig. 12), whereas estradiol-$17\beta$ did not.

mainly oxytocin (Adamsone et al., 1956; Lederis, 1961). In the present experiment, estradiol-17β enhanced the incorporation of ³H-leucine in the suprăoptic nucleus and the paraventricular nucleus, while progesterone enhanced the incorporation only in the suprăoptic nucleus. Stumpf (1968 b; 1971) reported that estradiol concentrates in neurons of the central-posterior portion of the paraventricular nuclei. Attramadal (1964) found the significant labelling of nerve cells in the suprăoptic and paraventricular nuclei of the hypothalamus after the injection of ³H-estradiol. König and Böttcher (1966) showed the highest content of oxytocin and vasopressin in these areas on the day of proestrus on which the concentration of estrogen in blood was very high (Hori et al., 1968). Fendler (1961) obtained the increased amount of oxytocin in the neurohypophysis of the rat after administration of estrogen. Yagimuma et al. (1969) found the higher uptake of ³H-leucine in the suprăoptic and paraventricular nuclei in normal rats than in castrated ones. These results indicate that estrogen concentrates in the paraventricular and suprăoptic nuclei and stimulates synthesis of oxytocin and vasopressin, which agree well with the present results obtained by a single injection of estradiol-17β. This experiment first demonstrates that protein synthesis is stimulated in the suprăoptic nucleus of progesterone-pretreated castrated rats. It is, however, not clear from this experiment whether progesterone directly affects this nucleus to produce oxytocin and/or vasopressin in the same manner as estradiol does. A further study should be made to clarify this problem.

In the castrated rats, a single injection of estradiol-17β did not enhance the incorporation of ³H-leucine in the cell bodies of neurons in the suprachiasmatic nucleus and the anterior periventricular nucleus located in the anterior hypothalamus, as compared with that in the castrated controls. On the other hand, a significant increase in the incorporation of the radioisotope was induced in the cell bodies of neurons in the arcuate nucleus and the ventromedial nucleus of the estradiol-17β-pretreated animals, suggesting an enhancement of protein synthesis in these hypothalamic regions. Although there seems no evidence showing that the administration of estrogen to the castrated animals augments gonadotrophin releasing factors in the hypothalamus, it is suggested that the regions are related to the regulation of pituitary-gonadal system through the release regulating center in the anterior hypothalamus (Piacsek and Meites, 1966; Flerkó, 1966; Gorski, 1966; Barraclough, 1967; Everett, 1969; Halász, 1969; Barraclough, 1970). In addition, estrogen sensitive receptor might be located in the anterior hypothalamic region, and a close relationship exists between the level of estrogen and the action of this part of hypothalamus in controlling gonadotrophic activity of the pituitary gland and ovulation (Szente-gothai et al., 1968 b). Taking these reports into consideration, the present result would suggest that at least the positive feedback action of estradiol-17β stimulating the arcuate and ventromedial nuclei to produce gonadotrophin releasing factors is mediated presumably through the release regulating center in the anterior hypothalamus, although there is no evidence that estradiol-17β has an effect directly on the anterior hypothalamic region, as far as this experiment is concerned.

A single injection of progesterone induced a significant increase in the incorporation of ³H-leucine in the cell bodies of neurons both in the anterior periventricular and suprachiasmatic nuclei and in the arcuate and ventromedial nuclei. It is well known that the physiological effect of progesterone in animals including man is revealed under the influence of estrogen, of which priming the animals with estrogen seems to be an important factor. For example, Nallar et al. (1967) found that ovariectomized rats given no estrogen prior to progesterone failed to respond to progesterone by a rise in plasma luteinizing hormone. On the contrary, Caligaris et al.,
(1968) obtained the beautiful biphasic pattern of plasma luteinizing hormone in ovariectomized estrogen-pretreated rats injected with progesterone, indicating that progesterone showed its physiological effect after priming with estrogen. In the present experiment, the castrated rats were not primed with estrogen. Therefore, although progesterone enhanced the incorporation of $^3$H-leucine in the cell bodies of neurons in the arcuate, ventromedial, anterior periventricular and suprachiasmatic nuclei, suggesting an increase in protein synthesis in these nuclear regions, it may not be said that this effect of progesterone is physiological. A further study to show a physiological effect of this hormone on the uptake of leucine in the brain of rats is in progress.

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References


