Effect of Sex Steroids on the Uptake of $^3$H-leucine by Brain Tissues of Ovariectomized Mice

Kanji SEIKI, Hideko FUJII and Mayumi NAKANO

Department of Anatomy, School of Medicine, Tokai University

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Effect of the sex steroids, progesterone (P) and testosterone (T), on $^3$H-leucine uptake by the brain cells of ovariectomized mice were examined. Animals were divided into four groups, i.e. group 1: control animals treated with sesame oil; group 2: animals treated with P; group 3: animals treated with T, and group 4: animals first treated with T and then with P. Animals in each group were given a single i.p. injection of $^3$H-leucine 24 hr after the last hormonal treatment, and sacrificed 2 hr later. Intensity of the uptake of the radiochemical was measured by counting the number of reduced silver grains over cell bodies in various brain regions using an autoradiographic technique.

Group 1 showed a relatively high uptake in the SO, PV and SPH when compared with that in the remaining nuclei examined. Groups 2 and 3 both showed a significant enhancement of the uptake in SCH, ARC and PM when compared with that in group 1. Group 4 showed enhancement of the uptake in most of the nuclei except POL, DM and SPH when compared with that in group 1. However, only the POM, PV, SO and VM revealed a significantly higher uptake than the respective nuclei in groups 2 and 3. The uptake by cells in the EC and CC remained unchanged after the hormonal treatment.

The present results suggest that in female mice P or T stimulates protein synthesis in the hypothalamic nuclei and that the effect of P on protein synthesis is greatly influenced by T-priming.

(Key Words: Hypothalamus, Progesterone, Testosterone, $^3$H-leucine, Autoradiography)

INTRODUCTION

Substantial data have accumulated to demonstrate estrogen (E) receptor (4, 6, 8, 12, 26), progesterone (P) receptor (9, 13, 19, 21) and androgen receptor (1, 2, 7, 11) in female brains of various species of animals. Steroid hormones enter the target cell membrane and bind to a cytosol receptor. The steroid-receptor complex then enters the cell nucleus where it interacts with DNA. This interaction may induce an alteration in the transcription of a specific gene resulting in the alteration of cellular function (25). Based on this concept, we have investigated the effect of E and/or P on the uptake of $^3$H-leucine into protein molecules by the brain tissues of female rats and mice (17, 18, 20). According to our data, both hormones have positive effects on the uptake of the radiochemical by most of the hypothalamic nuclei, and E-priming enhances the effect of P. In the present study, the effect of testosterone (T) and P, and a combination of both hormones on the uptake of $^3$H-leucine by various brain regions of castrated mice was examined using an autoradiographic technique.

Kanji Seiki, Department of Anatomy, School of Medicine, Tokai University, Bohseidai, Isehara, Kanagawa 259-11, Japan
MATERIALS AND METHODS

Adult female mice of the ICR strain, weighing about 25 g, where castrated 7 days prior to subsequent hormonal treatment. They were divided into four groups, each group consisting of three animals. Group 1 received s.c. injections of 0.05 ml of sesame oil once a day for 4 days. Groups 2 and 3 received s.c. injections of 0.1 mg of P and 0.1 mg of T, respectively, in 0.05 ml of sesame oil once a day for 4 days. Group 4 received s.c. injections of 0.1 mg of T in 0.05 ml of sesame oil once a day for the first 3 days. On the next day this group received a single s.c. injection of 0.1 mg of P in the oil. Twenty four hr after the last injection, all the animals in each group were given a single i.p. injection of [4,5-3H(N)]-L-leucine (sp. act. 60 Ci/mmol; New England Nuclear, Boston) dissolved in physiological saline at a dose level of 10 μCi/g of body weight. Two hr later the animals were killed by decapitation. The brains were carefully extirpated and dipped in 10% formaldehyde solution neutralized by MgCO₃.

Preparations of the tissue slices and autoradiographic procedures were performed as described previously (17). In the present study, slides coated in liquid emulsion (Sakura NR-M₂; Konishiroku Photo. Ind., Co., Tokyo) were exposed for 3 months. Localization of the brain regions was confirmed according to the atlas of the mouse brain (23). The uptakes of radioactive leucine and its derivatives in the cell bodies of neurons in the medial preoptic region (POM), the lateral preoptic region (POL), the suprachiasmatic nuclear region (SCH), the supraoptic nuclear region (SO), the middle part of the paraventricular nuclei (PV), the middle part of the periventricular arcuate nuclei (ARC), the anteromedial part of the ventromedial nuclei (VM), the dorsomedial nuclear region (DM), the stratum pyramidale hippocampi (SPH), the ventral part of the pre mammillary nuclei (PM), the cerebral cortex (CC) and the ependymal cells surrounding the third ventricle were measured by counting the number of reduced silver grains in the cell body in the same plane focus as the top of the tissue slice. The grains were counted from at least 100 cells in 15 slices for each brain region from three mice in each group. The average numbers of grains in each corresponding brain region were not statistically different between each animal in the same group. The average number of grains was, therefore, calculated by combining each grain number per unit cell in various regions of the brain in each group.

RESULTS

Table 1 shows the average number of reduced silver grains per cell in various brain regions in four groups of animals. In group 1, cell bodies of neurons in magnocellular nuclei, SO (Fig. 3), PV (Fig. 5) and SPH, showed a fairly high uptake of ³H-leucine when compared with that in the remaining cell groups. Among the remaining cells, the uptakes by the DM, PM (Fig. 11) and VM (Fig. 7) were at the same levels, which were about a half of those by the SO, PV and SPH. The nuclei in SCH and ARC (Fig. 9) showed a slightly higher uptake than that by the DM, PM and VM.
The uptake by cells in POM (Fig. 1), POL, EC and CC was the lowest among the brain regions examined.

In groups 2 and 3, the uptake of the radiochemical by the SCH, ARC and PM was slightly higher than that by the respective nuclei in group 1. However, the remaining cells showed almost the same uptake as that in group 1.

In group 4, a significantly high uptake of the radioactive leucine was seen in the cell bodies of the POM (Fig. 2), SCH, SO (Fig. 4), PV (Fig. 6), VM (Fig. 8), ARC (Fig. 10) and PM (Fig. 12) when compared with that in group 1. However, among these nuclei only the cell bodies of the POM, SO, PV and VM showed a slightly higher uptake than the respective cell nuclei in groups 2 and 3. The remaining brain cells in the POL, DM, EC and CC showed almost the same level of uptake as that in groups 1, 2 and 3.

Table 1  Distribution of radioactivity in cell bodies of various brain regions of ovariectomized mature ICR mice 2 hr after a single i.p. injection of $^3$H-leucine (10$\mu$Ci/g of body weight)

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Reduced silver grains/cell (Mean±S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1*</td>
</tr>
<tr>
<td>POM</td>
<td>7.4±0.3</td>
</tr>
<tr>
<td>POL</td>
<td>6.7±0.3</td>
</tr>
<tr>
<td>SCH</td>
<td>15.0±0.4</td>
</tr>
<tr>
<td>SO</td>
<td>19.7±0.6</td>
</tr>
<tr>
<td>PV</td>
<td>22.0±1.0</td>
</tr>
<tr>
<td>VM</td>
<td>13.9±0.3</td>
</tr>
<tr>
<td>DM</td>
<td>10.8±0.4</td>
</tr>
<tr>
<td>SPH</td>
<td>18.2±0.8</td>
</tr>
<tr>
<td>ARC</td>
<td>16.4±0.4</td>
</tr>
<tr>
<td>PM</td>
<td>13.0±0.4</td>
</tr>
<tr>
<td>EC</td>
<td>8.0±0.3</td>
</tr>
<tr>
<td>CC</td>
<td>6.4±0.3</td>
</tr>
</tbody>
</table>

* Details of the hormonal treatments in each group are described in Methods.
# P<0.005 in group 1 vs group 2, group 1 vs group 3 and group 1 vs group 4.
+ P<0.005 in group 2 vs group 4 and group 3 vs group 4.

DISCUSSION

The present study reveals that all the brain regions examined contained various degrees of reduced silver grains. This indicates that $^3$H-leucine was incorporated into protein molecules which had been newly synthesized within the cells after administration of the radiochemical.

Treatment of animals with P or T brought about an increased protein synthetic activity of the SCH and ARC. Sheridan et al (22) have shown the high uptake of T by these nuclei in female rats. Sar and Stumpf (15) have shown the high incorporation of P into these nuclei of female guinea pigs. Chamness et al (3) showed the existence of androgen receptors in
the hypothalamus containing the SCH and ARC, and the preoptic region of immature female rats. In addition, Schally et al (16) have reported that gonadotrophin releasing hormone (GRH) has a leucine fragment in its molecule. Taking these reports into consideration, the present results may indicate that the increased protein synthesis in these nuclei after treatment of animals with P or T is related to the increased synthesis of GRH in these nuclei as mentioned in the early study using E in female mice (20).

In the SCH and ARC, treatment of animal with T and P brought about protein synthesis at almost the same level but not a higher level than that after treatment with P or T. In our previous papers (18, 20),

Figs. 1-6 Autoradiographs of cell bodies of neurons in various brain regions in groups 1 and 4. 1. POM (group 1), 2. POM (group 4), 3. SO (group 1), 4. SO (group 4), 5. PV (group 1), 6. PV (group 4). Note more numerous silver grains in nuclei in group 4 than in group 1. Toluidine blue stain. ×1,200 for Figs. 1, 2, 5 and 6, and ×500 for Figs. 3 and 4.
E-priming increased protein synthesis by P in these nuclei of female rats and in the ARC of female mice. This may indicate that, unlike E-priming, T-priming does not enhance the effect of P on these nuclei to synthesize GRH.

In the SO and PV, T-priming enhanced the effect of P on protein synthesis whereas P and T did not. The molecular structure of oxytocin is fixed with leucine linked to the tripeptide tail (5). Kozlowski et al (10) have demonstrated that SO and PV are oxytocin-and vasopressin-containing neurons. The present results, therefore, seem to indicate that sequential treatment of animals with T and P stimulates these nuclei to produce oxytocin.
Finally, there is evidence that the hypothalamus is more active than the cortex in converting T to E (24). There is also evidence to show that induction of female sexual behavior and hypothalamic virilization is brought about after the conversion of T to E (14). It must, therefore, be kept in mind that some of the effects of T on the brain tissues of female animals, including synthesis of GRH and oxytocin, is mediated through the conversion of T to E.

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