Effects of Progesterone and Estrone on the Conversion of Tryptophan to Nicotinamide in Rats

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The effects of the ovarian hormones progesterone and estrone on the conversion of tryptophan to nicotinamide in rats were investigated. Female rats were fed for 35 days with a 20% casein diet, or with a 20% casein diet containing 0.1% progesterone, or 0.001% estrone, or 0.1% progesterone and 0.001% estrone. The conversion ratio of tryptophan to nicotinamide on the last day of the experiment was 2% in the groups fed with the 20% casein diet and the diet containing 0.1% progesterone, but around 1.2% in the group fed with 0.001% estrone, and 0.7% in the group fed with 0.1% progesterone and 0.001% estrone. These results demonstrated that administration of ovarian hormones significantly decreased the conversion of tryptophan to nicotinamide.

Miller\textsuperscript{11} drew attention to the fact that during the first half of this century, when pellagra was a major problem in the United States, deaths attributable to the disease were approximately twice as common among females than males. Since pellagra is caused by a deficiency of Nam, NIA, or Trp, these findings suggest that female hormones affect the metabolism of Trp into Nam. In fact, women using oral contraceptives containing synthetic estrogen and progestogen combinations excrete abnormally high levels of such Trp metabolites as xanthurenic acid, kynurenic acid, kynurenine, and 3-hydroxykynurenine after a loading dose of Trp.\textsuperscript{2-9} Bender and Totoe\textsuperscript{10} reported that the administration of estrone sulfate to rats led to significant inhibition of kynurenine 3-hydroxylase, kynureninase, and 3-hydroxyanthranilic acid oxygenase, and a significant reduction in the liver content of NAD and the urinary excretion of MNA. These reports suggest that ovarian hormones reduce the conversion of Trp into Nam. On the other hand, Lojkin \textit{et al.}\textsuperscript{11} reported that there was a marked increase in the urinary excretion of MNA by human subjects in pregnancy and there was a significant increase in the excretion of MNA in pregnant rats. Lojkin\textsuperscript{12} also reported that administration of progesterone and estrone to rats induced an increase in MNA excretion. Wertz \textit{et al.}\textsuperscript{13} reported the data obtained from a study of the excretion of Trp, MNA, 2-Py, and quinolinic acid in a group of women who consumed a supplement of Trp in the last trimester of pregnancy and again after the third month postpartum, indicated that the conversion of Trp to Nam is more efficient in the pregnant than in the nonpregnant state. However, no data have been reported that indicate how the administration of ovarian hormones affects the Trp to Nam conversion.

To examine the conversion of Trp to Nam, reliable methods are needed for measuring Nam and its metabolites such as MNA, 2-Py, and 4-Py. In 1988, we developed an assay for 4-Py, and found that the most abundant metabolite of Nam was 4-Py, which accounts for over 80%. Therefore, to calculate the conversion of Trp to Nam, the urinary excretion of 4-Py must be measured. In this paper, we investigated the effects of dietary progesterone and estrone on the conversion of Trp to Nam in rats.

Materials and Methods

\textbf{Chemicals.} NAD$^+$ was purchased from the Sigma Chemical Company (St. Louis, MO, U.S.A.). Vitamin-free milk casein, sucrose, l-methionine, Nam, anthranilic acid, l-Trp, progesterone, and estrone were purchased from Wako Pure Chemical Industries (Osaka, Japan). Kynurenine sulfate, kynurenic acid, 3-hydroxyanthranilic acid, and MNA chloride were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). 2-Py and 4-Py were synthesized by the methods of Pullman and Colowick\textsuperscript{14} and Shibata \textit{et al.}\textsuperscript{15} respectively. Gelatinized cornstarch and corn oil were purchased from Nichiden Kagaku (Tokyo, Japan) and Ajinomoto (Tokyo, Japan), respectively. The mineral and vitamin mixtures were obtained from Oriental Yeast Kogyo (Tokyo, Japan). All the other chemicals used were of the highest purity available from commercial sources.

\textbf{Animals and diets.} Female rats of the Wistar strain (7 weeks old) were obtained from Clea Japan (Tokyo, Japan). The rats were immediately placed in individual metabolism cages (CT-10; obtained from Clea Japan). To accustom the rats to these conditions, they were initially fed with an NIA-free, 20% casein diet (Table I) for 14 days \textit{ad libitum}. They were then divided into four groups and fed for 35 days with the NIA-free, 20% casein diet, or the same diet +0.1% progesterone, or 0.001% estrone, or 0.1% progesterone +0.001% estrone (Table I). The contents of estrone in the diet was determined by the method in ref. 10, and that of progesterone was determined by assuming that the ratio of progesterone/estrone is about 100, as it is in human corpus luteum.\textsuperscript{16}

The room temperature was maintained at 22 ± 2°C and about 60% humidity and a 12-h light/12-h dark cycle was maintained. Body weight and food intake were measured daily at around 09:00 a.m. Urine samples (24-h, 09:00 a.m.-09:00 a.m.) were periodically collected in amber bottles containing 1 ml of 1 M HCl, and were stored at -25°C until needed. The rats were killed by decapitation at around 09:00 a.m. on the last day of the experiment. A 10-μl sample of blood was taken from the carotid artery and treated as described in the literature\textsuperscript{17} for measuring NAD (NAD$^+$ + NADH). The livers were removed, and a portion of the liver (approximately 0.2 g) was immediately treated as described in the literature\textsuperscript{17} for measuring NAD. Another portion of the liver (approximately 1 g) was treated as described in the literature\textsuperscript{18,19} for measuring the enzyme activities involved in metabolism of Trp to Nam.

\textbf{Analyses.} Trp oxygenase (EC 1.13.11.11),\textsuperscript{20} kynureninase (EC 3.7.1.3),\textsuperscript{20} kynurenine aminotransferase (EC 2.6.1.7),\textsuperscript{21} 3-hydroxy-

\textbf{Abbreviations:} Nam, nicotinamide; NIA, nicotinic acid; Trp, tryptophan; MNA, N$^1$-methylnicotinamide; 2-Py, N$^1$-methyl-2-pyridone-5-carboxamide; 4-Py, N$^1$-methyl-4-pyridone-3-carboxamide.
Table I. Composition of Diets

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>+0.1% Pro</th>
<th>+0.001% Est</th>
<th>+0.1% Pro</th>
<th>+0.001% Est</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin-free</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>milk casein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>l-Methionine</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Gelatinized</td>
<td>45.9</td>
<td>45.8</td>
<td>45.899</td>
<td>45.789</td>
<td></td>
</tr>
<tr>
<td>cornstarch</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>22.9</td>
<td>22.9</td>
<td>22.9</td>
<td>22.9</td>
<td></td>
</tr>
<tr>
<td>Corn oil</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>(Oriental’s ratio)*</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>(NIA-free)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Progesterone</td>
<td>0</td>
<td>0</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Estrone</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The composition of the minearl and vitamin mixtures are described in ref. 17.

anthranilic acid oxygenase (EC 1.13.11.6),(20) aminocarboxyamonoacetamidodehydro decarboxylase (ACMSDase, EC 4.1.1.45),(21) nicotinamide methyltransferase (EC 2.1.1.1),(20) 2-Py-forming MNA oxidase (EC 2.1.3.1),(21) and 4-Py-forming MNA oxidase (EC number not determined)(23) were measured as described in the literature.

To calculate the conversion ratio of Trp to Nam, the urinary contents of Nam and the metabolites MNA, 2-Py, and 4-Py were measured. The conversion ratio was calculated as the sum of the urinary excretions of Nam+MNA+2-Py+4-Py (μmol/day) × 100/Trp intake (μmol/day). This method does not take into account the content of Nam in the body weight gain, and the conversion ratio does not, therefore, mean the net conversion ratio. The contents of Nam, 2-Py, and 4-Py in the urine were simultaneously measured by the HPLC method of Shibata et al.(19) while the content of MNA in the urine was measured by the HPLC method of Shibata.(24) The content of NAD (NAD+ + NADH) was measured by the colorimetric method of Shibata and Murata.(17)

Results

Body weight and food intake

The daily body weight changes in the four groups are shown in Fig. 1. The gain in body weight and food intake were not affected by feeding the diet containing progesterone but were significantly decreased by feeding the diet containing estrone, as shown in Table II. These values were further decreased by feeding the diet containing both progesterone and estrone.

Enzyme activities involved in Trp to Nam metabolism

The activity of Trp oxygenase was higher in the group fed progesterone and estrone than in the other groups, and that of ACMSDase was also higher in the group fed both hormones than in the group fed with the hormone-free diet, as shown in Table III. The activities of kynureninase, kynurenine aminotransferase, 3-hydroxyanthranilic acid oxygenase, Nam methyltransferase, and 2-Py-forming MNA oxidase did not vary significantly between any of the four groups. The activity of 4-Py-forming MNA oxidase was lower in the progesterone and estrone group than in the other three groups.

The contents of NAD in blood, liver, and ovary

The contents of NAD in blood, liver, and ovary did not vary between any of the four groups under these experimental conditions (Table IV).

The contents of MNA, 2-Py, and 4-Py in liver

The contents of MNA in the liver did not vary between any of the four groups and the contents of 2-Py and 4-Py in the liver were below the limit of detection (Table V).

Urinary excretion of Trp metabolites

The urinary excretion of the Trp metabolites as kynurenic acid, anthranilic acid, xanthurenic acid, 3-hydroxyanthranilic acid, Nam, MNA, 2-Py, and 4-Py is shown in Table VI. The anthranilic acid excretion was higher in the group fed both hormones than in the control group. The kynurenic acid, xanthurenic acid, and 3-hydroxyanthranilic acid excretion did not vary between any of the four groups. The excretion of Nam, MNA, 2-Py, and 4-Py was significantly lower in the group fed both hormones and slightly lower in the group fed estrone than in the control group.

Table II. Effects of Progesterone (Pro) and Estrone (Est) on the Body Weight Gain, Food Intake, and Food Efficiency Ratio (FER=Body Weight Change/Food Intake)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>+0.1% Pro</th>
<th>+0.001% Est</th>
<th>+0.1% Pro</th>
<th>+0.001% Est</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
<tr>
<td>Initial body weight (g)</td>
<td>177 ± 3</td>
<td>176 ± 2</td>
<td>174 ± 2</td>
<td>173 ± 3</td>
<td></td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>221 ± 4x</td>
<td>220 ± 3x</td>
<td>197 ± 5x</td>
<td>173 ± 3x</td>
<td></td>
</tr>
<tr>
<td>Body weight gain (g/35 days)</td>
<td>45 ± 2x</td>
<td>43 ± 1x</td>
<td>23 ± 4x</td>
<td>-0.05 ± 2.0x</td>
<td></td>
</tr>
<tr>
<td>Food intake (g/35 days)</td>
<td>456 ± 12x</td>
<td>480 ± 7x</td>
<td>376 ± 14x</td>
<td>306 ± 9x</td>
<td></td>
</tr>
<tr>
<td>Trp intake (g/35 days)</td>
<td>1.03 ± 0.03x</td>
<td>1.09 ± 0.02x</td>
<td>0.85 ± 0.02x</td>
<td>0.69 ± 0.02x</td>
<td></td>
</tr>
<tr>
<td>Food efficiency ratio (body weight gain/food intake)</td>
<td>0.098 ± 0.003x</td>
<td>0.094 ± 0.006x</td>
<td>0.081 ± 0.022x</td>
<td>0.006 ± 0.016x</td>
<td></td>
</tr>
</tbody>
</table>

* Trp intake (g)=food (g) × 0.2 (casein content) × 0.869 (protein content in casein) × 0.013 (Trp content in casein). Values are means ± SEM for five rats; Values with different superscript letters in the same row are significantly different at p<0.01 as determined by Duncan’s new multiple-range test.
Table III. Effects of Progesterone (Pro) and Estrone (Est) on the Enzyme Activities Involved in Trp to Nam Metabolism

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>+0.1%Pro</th>
<th>+0.001%Est</th>
<th>+0.1%Pro</th>
<th>+0.001%Est</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver weight (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.90 ± 0.18</td>
<td>7.94 ± 0.30</td>
<td>6.85 ± 0.26</td>
<td>6.42 ± 0.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tryptophan oxidase</td>
<td>910 ± 38</td>
<td>987 ± 43</td>
<td>946 ± 78</td>
<td>1422 ± 115</td>
<td></td>
</tr>
<tr>
<td>(nmol/h/g of liver)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kynureninase</td>
<td>1271 ± 71</td>
<td>1276 ± 38</td>
<td>1363 ± 53</td>
<td>1390 ± 58</td>
<td></td>
</tr>
<tr>
<td>(nmol/h/g of liver)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kynurenin aminotransferase</td>
<td>1109 ± 85</td>
<td>1079 ± 34</td>
<td>1183 ± 30</td>
<td>1096 ± 32</td>
<td></td>
</tr>
<tr>
<td>(nmol/h/g of liver)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-Hydroxyanthranilic acid oxidase</td>
<td>595 ± 19</td>
<td>575 ± 31</td>
<td>520 ± 12</td>
<td>548 ± 30</td>
<td></td>
</tr>
<tr>
<td>(nmol/mmol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACMSDase</td>
<td>0.93 ± 0.16</td>
<td>1.20 ± 0.13</td>
<td>1.46 ± 0.25</td>
<td>2.00 ± 0.21</td>
<td></td>
</tr>
<tr>
<td>(nmol/mmol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nicotinamide methyltransferase</td>
<td>534 ± 40</td>
<td>507 ± 21</td>
<td>523 ± 21</td>
<td>482 ± 16</td>
<td></td>
</tr>
<tr>
<td>(nmol/mmol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Py-forming MNA oxidase</td>
<td>379 ± 42</td>
<td>377 ± 36</td>
<td>387 ± 40</td>
<td>318 ± 32</td>
<td></td>
</tr>
<tr>
<td>(nmol/mmol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Py-forming MNA oxidase</td>
<td>680 ± 45a</td>
<td>606 ± 33</td>
<td>531 ± 61</td>
<td>393 ± 14b</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SEM for five rats; Values with different superscript letters in the same row are statistically different at p<0.01 as determined Duncan’s new multiple-range test.

Table IV. Effects of Progesterone (Pro) and Estrone (Est) on the Contents of NAD in Blood, Liver, and Ovary

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>+0.1%Pro</th>
<th>+0.001%Est</th>
<th>+0.1%Pro</th>
<th>+0.001%Est</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood NAD (nmol/ml)</td>
<td>56.7 ± 2.4</td>
<td>54.5 ± 1.7</td>
<td>51.6 ± 1.6</td>
<td>52.2 ± 2.7</td>
<td></td>
</tr>
<tr>
<td>Liver NAD (nmol/g)</td>
<td>652 ± 36</td>
<td>627 ± 35</td>
<td>587 ± 28</td>
<td>605 ± 38</td>
<td></td>
</tr>
<tr>
<td>Ovary NAD (nmol/g)</td>
<td>268 ± 8</td>
<td>248 ± 21</td>
<td>243 ± 16</td>
<td>229 ± 33</td>
<td></td>
</tr>
</tbody>
</table>

Values and means ± SEM for five rats.

Table V. Effects of Progesterone (Pro) and Estrone (Est) on the Contents of MNA, 2-Py, and 4-Py in Liver

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>+0.1%Pro</th>
<th>+0.001%Est</th>
<th>+0.1%Pro</th>
<th>+0.001%Est</th>
</tr>
</thead>
<tbody>
<tr>
<td>MNA (nmol/g of liver)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>71 ± 3</td>
<td>72 ± 3</td>
<td>67 ± 2</td>
<td>71 ± 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Py (nmol/g of liver)</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>4-Py (nmol/g of liver)</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SEM for five rats. N.D. = not detected.

The excretion ratio of (2-Py + 4-Py)/MNA was lower in the progesterone and estrone group than in the other three groups.

Trp to Nam conversion
As shown in Fig. 2, the conversion ratio in the group fed the NiA-free, 20% casein diet remained around 2% during the experimental period. It was not affected by the feeding of progesterone alone but was decreased by the feeding of

Fig. 2. Effects of Progesterone and Estrone on the Conversion of Trp to Nam.
- 20% casein diet (control diet); O, 20% casein diet +0.1% progesterone; [ ], 20% casein diet +0.001% estrone; -[ ], 20% casein diet +0.1% progesterone +0.001% estrone. Each point represents the mean ± SEM for five rats; Points with different superscript letters on the same day are significantly different at p<0.01 as determined by Duncan’s new multiple range test.

Discussion
Lojkine and colleagues10–13) reported that the administration of female hormones induced an increased excretion of MNA; female hormones stimulate the conversion of Trp to Nam. We here measured the excretions of 2-Py and 4-Py to investigate whether the high levels of MNA excretion reported by Lojkine and colleagues10–13) might be due to an increase of these metabolites in the tissues because of impairment of the oxidizing processes which convert MNA into 2-Py and 4-Py. Our data showed no increase in urinary

estrone and markedly decreased by the combination of progesterone and estrone.
excretion of MNA in rats fed with the diet containing estrone and progesterone compared with the control group, and no significant difference in the contents of MNA in the liver between any of the four groups. However, the activity of 4-Py-forming MNA oxidase was significantly lower in the estrone and progesterone group than in the other three groups (Table III) and the excretion ratio of (2-Py+4-Py)/MNA, which reflects the in vivo activity of the oxidizing processes, was also lower (Table VI). These results mean that when rats are fed with a diet containing sufficient NiA or Nam, the urinary excretion of MNA increases. In these experiments, the diets used were NiA-free and, consequently, no significant increase in MNA excretion would be expected.

Other investigators\(^2\) to \(^9\) have reported that female hormones induced increased excretion of such by-products of the conversion of Trp to Nam as xanthurenic acid, kynurenic acid, and 3-hydroxykynurenine after a loading dose of Trp, which implies that administration of female hormones decreases the conversion of Trp to Nam. In our data, however, although the activity of Trp oxygenase was higher in the group fed with the diet containing both hormones than in the other three groups (Table III), no increase was observed in urinary excretion of xanthurenic acid, kynurenic acid, or 3-hydroxyxanthanilic acid (Table VI). This difference appears to be attributable to the experimental methods: while others\(^2\) to \(^9\) administered a loading dose of Trp to rats, we did not. Bender and Toteo\(^10\) reported that the administration of estrone sulfate to rats led to significant inhibition of kynureninase and 3-hydroxyxanthanilic acid oxygenase and a significant reduction in the liver content of NAD. But our data were not consistent with their results; these enzyme activities were not affected by the hormones (Table III) and the liver NAD content did not decrease. The reason for this disagreement is not clear.

The effects of ovarian hormones on the conversion of Trp to Nam have not been reported. In our data, the Trp to Nam conversion was decreased to about one-third of that of the control group (Fig. 2). This demonstrates that the increases in progesterone and estrone in body tissues at such times as pregnancy and the postpartum phase induce the decrease in the conversion of Trp to Nam. It also suggests that the twofold incidence of pellagra deaths in women is attributable to the female hormones. The mechanism by which the female hormones lead to a decrease in Trp to Nam conversion may involve the increased activity of ACMSDase (Table III). Quinolinic acid, which is a very important intermediate in the conversion of Trp to Nam, is formed nonenzymatically from \(\alpha\)-amino-\(\beta\)-carboxymuconate-\(\epsilon\)-semialdehyde (ACMS). ACMS is catalyzed enzymatically by ACMSDase to \(\alpha\)-aminomuconate-\(\epsilon\)-semialdehyde (AMS) which is then catabolized into acetyl-CoA. Therefore, increased activity of ACMSDase may lead to the decreased formation of quinolinic acid. It is reported that the ACMSDase activity is subject to many factors such as protein intake\(^18\) to \(^27\) fat intake\(^28\) to \(^29\) and hormones\(^30\) to \(^34\). Therefore, this enzyme may play a critical role in the conversion of Trp to Nam, but the mechanism by which these factors affect the enzyme protein synthesis is not clear.

References