Accumulation of Brain Tryptophan in Rats after Administering Various Fats or Fatty Acids

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The intragastric administration of various kinds of fat (corn oil, lard, safflower oil, perilla oil, and fish oil) or fatty acids (oleic acid, linoleic acid, linolenic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA)) caused a significant increase in brain tryptophan (especially with fish oil or DHA), and also in serum insulin. However, at 2 h after administering the fats or fatty acids, brain serotonin was not increased in accordance with the increase of brain tryptophan. As a result of determining the time-dependent changes in brain 5-hydroxyindoles, brain serotonin and 5-HIAA gradually increased, and showed significant increases at 6 h and 4 h after the administration of fish oil, respectively. These results suggest that there was a fairly long time-lag for the changes in brain 5-hydroxyindoles to appear after being induced by the administration of fats or fatty acids.

Changes in the dietary composition (carbohydrate and/or protein, amino acids or some xenobiotics) affect the level of serotonin, a putative neurotransmitter, in the mammalian central nervous system.1–6 This serotonin synthesis is mainly regulated by the supply of tryptophan, a precursor for serotonin synthesis, to the brain via the blood-brain barrier, and tryptophan transport into the brain is controlled by competitive inhibition by other large neutral amino acids (LNAA) such as tyrosine, phenylalanine, and branched-chain amino acids.7,8 Circulating tryptophan in the blood is bound to albumin,9 and its binding is inversely related to the plasma free fatty acid level,10 because free fatty acid is also transported in the albumin-bound form in blood. Therefore, dietary fat might alter the balance between the free and albumin-bound forms of tryptophan, and consequently affect the incorporation of tryptophan into the brain and the synthesis of serotonin. On the other hand, the lipid concentration in the brain is high, especially that of the polyunsaturated fatty acids of phospholipids. It has recently been reported that the proportion of linoleic acid (n-6) and linolenic acid (n-3) in the diet affected memory behavior in the brightness-discrimination learning test: the correct response ratios were higher in the perilla oil group than in the safflower oil group.11 Another memory study has shown the different effects of soy oil and safflower oil.12

In this study, we examined whether various kinds of fats or fatty acids might affect the concentrations of brain tryptophan and 5-hydroxyindoles, and we observed that there were some differences in the degree of effect caused by the different fats or fatty acids, as well as a time-lag in the changes in brain tryptophan and serotonin.

Materials and Methods

Chemicals. Tryptophan, serotonin, 5-HIAA and fatty acids (with the exception of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which were obtained from Yaizu Suisankagaku Ind. Co., Shizuoka, Japan) were purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents such as various fats were purchased from Kanto Chemical Co. (Tokyo, Japan).

Experimental procedure. Young adult male rats1 of the Wistar strain were fed on a 20% casein diet for 1 week for adaptation. The basal diet (20% casein) contained, by weight, 20% casein, 68.7% carbohydrate (corn starch : sucrose = 2:1, w/w), 5% corn oil, 5% mineral mixture,2 1% vitamin mixture,2 and 0.15% choline-CI. On the day of the substance administration, the diet was removed at 7 a.m., and the test fats or fatty acids were given by intragastric intubation at 1 p.m. In experiment 1, the animals received perilla oil (1 ml per 100 g of body weight) and were killed by decapitation 1, 2, 3.5, and 5 h after administration. In experiment 2, the animals received various fats (corn oil, lard, safflower oil, perilla oil, or fish oil (Thunnus alalunga) : 1 ml per 100 g body weight, respectively) and were killed 2 h later. In experiment 3, the animals were treated with fatty acids (oleic acid, linoleic acid, linolenic acid, EPA, or DHA) and were then killed by decapitation after 2 h of treatment. In experiment 4, the animals received fish oil (1 ml per 100 g of body weight) and were killed 1, 2, 3, 4, and 5 h after administration. The brain was immediately removed, frozen on dry ice and stored at −70°C until the assay. Blood was collected from the cervical wound, and serum samples were stored at −20°C until the assay. Ammonia acid concentrations in the serum were determined with an amino acid autoanalyzer.2 The concentration of brain tryptophan was determined by the method of Denckla and Dewey,13 and brain serotonin and 5-HIAA were assayed fluorimetrically.14 Serum insulin concentrations were measured by a radioimmunoassay, using antibody coated beads.2

Statistical analysis. The statistical significance of the differences between values was determined by an analysis of variance and by Student’s t-test.15 p-Values of less than 0.05 were considered to indicate statistical significance.

Results

Time-dependent changes in brain tryptophan after intragastric administration of perilla oil in rats (Experiment 1)

The administration of perilla oil caused a significant rise in the brain tryptophan concentrations 1 and 2 h later, and...
thereafter, its content gradually decreased and returned to the control level (Table I). Therefore, 2h after administration was considered an adequate time to proceed to the analysis in subsequent experiments.

**Effect of intragastric administration of various fats on tryptophan in the serum and brain, and on brain 5-hydroxyindoles in rats (Experiment 2)**

The administration of corn oil, lard, perilla oil, or fish oil caused a significant increase in serum tryptophan (Table II). The tryptophan ratio (the ratio of the serum tryptophan concentration to the summed concentrations of the other large neutral amino acids) and serum insulin were also increased by the administration of various fats. The administration of all test fats caused a significant increase in brain tryptophan, especially of fish oil. However, the concentration of brain serotonin was not changed or tended to decrease by the administration of fats, with the exception of fish oil. The brain 5-HIAA concentration was increased by the administration of corn oil or lard, but the other fats did not affect its concentration.

### Table II. Effect of Various Oils on Brain Tryptophan, Serotonin, and 5-Hydroxyindole Acetic Acid, on Serum Insulin and Tryptophan, and on Tryptophan/LNAA\(^1\) Ratio in Rats\(^2\) (Experiment 2)

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Serum Insulin (µg/ml)</th>
<th>Serum Tryptophan (µg/ml)</th>
<th>Tryptophan/ LNAA(^1) (x10(^{-3}))</th>
<th>Brain Tryptophan (µg/g)</th>
<th>Brain Serotonin (ng/g)</th>
<th>Brain 5-HIAA(^3) (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Saline</td>
<td>15.7±1.2(^4)</td>
<td>5.19±0.27</td>
<td>13.7±1.4</td>
<td>4.2±0.5</td>
<td>457±17</td>
<td>393±9</td>
</tr>
<tr>
<td>2</td>
<td>Corn oil</td>
<td>18.8±1.7</td>
<td>6.44±0.11**</td>
<td>17.8±0.5</td>
<td>4.9±0.4*</td>
<td>462±24</td>
<td>438±15**</td>
</tr>
<tr>
<td>3</td>
<td>Lard</td>
<td>20.6±1.0*</td>
<td>6.67±0.28**</td>
<td>16.8±1.3</td>
<td>4.9±0.3*</td>
<td>454±14</td>
<td>444±12**</td>
</tr>
<tr>
<td>4</td>
<td>Safflower oil</td>
<td>20.6±2.5</td>
<td>5.27±0.19</td>
<td>15.8±0.5</td>
<td>4.8±0.3*</td>
<td>431±23</td>
<td>410±20</td>
</tr>
<tr>
<td>5</td>
<td>Perilla oil</td>
<td>21.1±1.3*</td>
<td>6.55±0.39</td>
<td>16.6±0.8</td>
<td>5.3±0.3***</td>
<td>435±20</td>
<td>416±9</td>
</tr>
<tr>
<td>6</td>
<td>Fish oil</td>
<td>20.1±0.6**</td>
<td>6.32±0.28*</td>
<td>16.8±1.4</td>
<td>5.7±0.5***</td>
<td>413±8</td>
<td>400±13</td>
</tr>
</tbody>
</table>

\(^1\) LNAA: Sum of 6 amino acids (Tyr, Val, Leu, Ile, Met, Phe).
\(^2\) The average body weight was 136±3 g.
\(^3\) 5-HIAA: 5-hydroxyindole acetic acid.
\(^4\) Means±SEM for five or six rats per group. *p<0.05, **p<0.01, ***p<0.001, compared with the saline value within a column (Student’s t-test).

### Table III. Effect of Various Fatty Acids on Brain Tryptophan, Serotonin, and 5-Hydroxyindole Acetic Acid, on Serum Insulin and Tryptophan, and on Tryptophan/LNAA\(^1\) Ratio in Rats\(^2\) (Experiment 3)

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Serum Insulin (µg/ml)</th>
<th>Serum Tryptophan (µg/ml)</th>
<th>Tryptophan/ LNAA(^1) (x10(^{-3}))</th>
<th>Brain Tryptophan (µg/g)</th>
<th>Brain Serotonin (ng/g)</th>
<th>Brain 5-HIAA(^3) (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Saline</td>
<td>15.6±1.4*</td>
<td>4.31±0.09</td>
<td>14.5±0.3</td>
<td>3.8±0.1</td>
<td>488±8</td>
<td>430±16</td>
</tr>
<tr>
<td>2</td>
<td>Oleic acid</td>
<td>21.3±0.5**</td>
<td>5.89±0.35**</td>
<td>18.2±0.8*</td>
<td>4.4±0.2*</td>
<td>494±8</td>
<td>519±18**</td>
</tr>
<tr>
<td>3</td>
<td>Linoleic acid</td>
<td>23.4±1.8**</td>
<td>5.68±0.59</td>
<td>16.7±0.5*</td>
<td>4.4±0.2*</td>
<td>493±16</td>
<td>524±16**</td>
</tr>
<tr>
<td>4</td>
<td>Linolenic acid</td>
<td>22.5±1.3**</td>
<td>6.54±0.09***</td>
<td>16.9±1.0</td>
<td>4.5±0.3</td>
<td>466±14</td>
<td>506±18*</td>
</tr>
<tr>
<td>5</td>
<td>EPA(^4)</td>
<td>24.7±2.9*</td>
<td>5.54±0.58</td>
<td>15.9±0.1*</td>
<td>4.7±0.1**</td>
<td>454±10</td>
<td>474±11</td>
</tr>
<tr>
<td>6</td>
<td>DHA(^3)</td>
<td>19.8±1.9</td>
<td>6.30±0.19**</td>
<td>19.4±2.1</td>
<td>4.8±0.2**</td>
<td>432±14**</td>
<td>466±21</td>
</tr>
<tr>
<td>7</td>
<td>DHA oil</td>
<td>19.0±1.1</td>
<td>5.13±0.05***</td>
<td>17.0±1.2</td>
<td>5.0±0.2**</td>
<td>424±14**</td>
<td>411±12</td>
</tr>
</tbody>
</table>

\(^1\) LNAA: Sum of 6 amino acids (Tyr, Val, Leu, Ile, Met, Phe).
\(^2\) The average body weight was 148±4 g.
\(^3\) 5-HIAA: 5-hydroxyindole acetic acid.
\(^4\) EPA: 5, 8, 11, 14, 17-eicosapentaenoic acid.
\(^3\) DHA: 4, 7, 10, 13, 16, 19-docosahexaenoic acid.
\(^*\) Means±SEM for six rats per group. *p<0.05, **p<0.01, ***p<0.001, compared with the saline value within a column (Student’s t-test).
various fatty acids, especially with EPA or DHA. However, the concentration of brain serotonin tended to decrease or was significantly decreased by the administration of fatty acids. The concentration of brain 5-HIAA was significantly increased by the administration of oleic, linoleic or linolenic acid.

**Time-dependent changes in brain 5-hydroxyindoles after intragastric administration of fish oil in rats (Experiment 4)**

The administration of fish oil did not change the level of serum tryptophan, but the brain tryptophan concentration was significantly high 1 h after treatment, and gradually decreased thereafter; however, 2 h after administration, it was still significantly high as compared with that before administration (Table IV). On the other hand, brain serotonin and 5-HIAA were significantly decreased in the first hour after oil administration, and thereafter gradually increased. Brain serotonin and 5-HIAA were significantly increased 6 and 4 h after treatment, respectively.

**Discussion**

The synthesis and release of neurotransmitters may regulate some important behavioral mechanisms such as the control of appetite, sleep, and so on. Brain serotonin is one of these neurotransmitters, and its concentration in the brain is affected by some dietary factors. For example, insulin or a high-carbohydrate meal elevated the brain tryptophan and 5-hydroxyindole levels. Insulin plays an important role in the change in concentration of brain 5-hydroxyindoles by accelerating the amino acid utilization for protein synthesis in peripheral tissues. On the other hand, dietary fat or fatty acid also affect the different responses of some behavior, as was observed in the brightness-discrimination learning test, X maze test and Morris water maze test; for example, soybean oil improved the performance with an environmentally-ecued testing paradigm which is thought to reflect cognitive learning skills (i.e., Place Navigation Water Task), or in the discrimination-learning test, the result of rats treated with linoleic acid (contained in soybean oil) being superior to that of rats fed with safflower oil. However, in these behavioral tests, brain neurotransmitters were not determined; in spite of the fact that there is a correlation between the serotonin concentration (one of the factors that controls some behavior) and memory learning ability.

In this study, the effects of fats (Table II) and fatty acids (Table III) on brain 5-hydroxyindoles were investigated. The administration of fish oil induced an increase in brain tryptophan, and on the contrary, decreased brain serotonin. As fish oil contains a high proportion of EPA or DHA, brain 5-hydroxyindoles were determined by the intragastric administration of EPA, DHA, or DHA oil (containing 25% of DHA). After each administration, brain tryptophan significantly increased and serotonin decreased in the same way as that with fish oil administration (5-HIAA did not show any significant changes). Therefore, EPA or DHA might change the concentrations of brain tryptophan and serotonin by reducing serotonin synthesis, accelerating serotonin degradation, or both, because recently, DHA has been shown to be one of the major unsaturated fatty acids of phospholipids in the brain and to be concerned in brain functions. As linoleic acid is the precursor of DHA, the effect of administering perilla oil (containing 65% of linoleic acid) or linoleic acid on brain 5-hydroxyindoles was examined. Perilla oil and linoleic acid each significantly increased or tended to increase brain tryptophan, but did not change brain serotonin. It is unclear why the administration of linoleic acid or DHA caused these different effects on brain serotonin. Corn oil (containing 50.2% of linoleic acid), safflower oil (containing 77.9% of linoleic acid) and linoleic acid caused a significant increase in brain tryptophan, but did not change the serotonin concentration (however, as 5-HIAA was increased by the administration of corn oil and linoleic acid, it might be considered that the degradation of serotonin was accelerated or the release of 5-HIAA was suppressed). Lard (containing 45.6% of oleic acid) and oleic acid caused significant increases in brain tryptophan and 5-HIAA; therefore, the overall synthesis of brain 5-hydroxyindoles might have been increased. In general, by 2 h after the administration of various kinds of fat or fatty acid, the serum tryptophan ratio and brain tryptophan were increased in almost all cases, but the effect on the concentrations of brain serotonin and 5-HIAA were different in each case. The reasons or mechanisms for these different responses of brain

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**Table IV. Time-dependent Changes in Brain Tryptophan, Serotonin, and 5-Hydroxyindole Acetic Acid after Administering Fish Oil to Rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Sacrifice time after treatment (h)</th>
<th>Serum tryptophan (μg/ml)</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tryptophan (μg/g)</td>
<td>Serotonin (ng/g)</td>
</tr>
<tr>
<td>1</td>
<td>None</td>
<td>0</td>
<td>3.3 ± 0.1</td>
<td>488 ± 3</td>
</tr>
<tr>
<td>2</td>
<td>Fish oil</td>
<td>1</td>
<td>5.6 ± 0.2**</td>
<td>409 ± 17*</td>
</tr>
<tr>
<td>3</td>
<td>Fish oil</td>
<td>2</td>
<td>4.6 ± 0.1**</td>
<td>475 ± 13</td>
</tr>
<tr>
<td>4</td>
<td>Fish oil</td>
<td>3</td>
<td>4.7 ± 0.1**</td>
<td>477 ± 9</td>
</tr>
<tr>
<td>5</td>
<td>Fish oil</td>
<td>4</td>
<td>4.1 ± 0.1**</td>
<td>491 ± 6</td>
</tr>
<tr>
<td>6</td>
<td>Fish oil</td>
<td>5</td>
<td>3.9 ± 0.1*</td>
<td>504 ± 14</td>
</tr>
<tr>
<td>7</td>
<td>Fish oil</td>
<td>6</td>
<td>3.6 ± 0.2</td>
<td>525 ± 4**</td>
</tr>
</tbody>
</table>

1 The average body weight was 130 ± 4 g.
2 5-HIAA: 5-hydroxyindole acetic acid.
3 Means ± SEM for five rats per group. Means within a column with an asterisk are significantly different from group 1 by Student's t-test (p < 0.05, **p < 0.01).
5-hydroxyindoles are not presently known. Also, it is not clear why the administration of fish oil did not change brain serotonin, in spite of the increase in brain tryptophan. The time-dependent responses of brain tryptophan and 5-hydroxyindoles by the administration of fish oil were then examined (Table IV), because, in experiments 2 and 3, these brain chemicals were determined at only one point, i.e., 2 h after the fat or fatty acid administration. At 2 h after the administration of fish oil, brain tryptophan indeed significantly increased, but brain 5-hydroxyindoles were not changed at this time (as shown in Table II). However, after this 2 h period, brain serotonin and 5-HIAA were gradually increased by the administration of fish oil, and had significantly increased at 6 h and 4 h, respectively (there was a time-lag in the increase of brain 5-hydroxyindoles).

The precise reason why the concentration of brain tryptophan was increased by the administration of a fat or fatty acid is not known. Fernstrom et al. have reported that dietary fat increased serum free fatty acid and thereby influenced the distribution of tryptophan in the free and albumin-bound forms (this is because approximately 80–90% of the total circulating tryptophan is bound to albumin). Therefore, we speculate that a free fatty acid was competing with tryptophan in albumin-binding, and when the rats were exposed to a fat or fatty acid, the albumin was used to bind the free fatty acid, and free tryptophan increased. The increase in brain tryptophan did not always match the time of the increase in brain serotonin synthesis. In this study, the mechanism for time-delayed changes in brain 5-hydroxyindoles and the direct relationship between fat or fatty acid and brain 5-hydroxyindoles were not determined. As fat, and especially polyunsaturated fatty acid, is one of the important constituents in the brain, further investigations concerning fat intake and brain components (or brain function in some cases) may be required.

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References