The roles of dietary tryptophan (Trp) were evaluated in regulation of production of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin (NT)-3 in the various brain regions in ddY mice. Feeding the mice a Trp-deficient diet for 2 weeks significantly decreased in the hippocampal level of NGF but not those of BDNF and NT-3, as compared with feeding an adequate Trp diet. The mice fed excess Trp did not have different levels of any of these neurotrophins than in the mice fed an adequate Trp diet. The levels of BDNF in the cerebral cortex were also significantly lower in the mice fed on a Trp-deficient diet, while the levels of NGF and NT-3 in the region were not modulated upon feeding of the diet. The dietary Trp level had no significant effect on the levels of NGF, BDNF, or NT-3 in the entorhinal cortex nor septum of the mice. These results demonstrate that the brain levels of NGF and BDNF are dependent on the dietary content of tryptophan.

Key words: Alzheimer’s diseases; tryptophan; nerve growth factor; brain derived neurotrophic factor; astrocytes

Nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) stimulate differentiation and/or growth of basal forebrain cholinergic neurons,1,2 loss of which is believed to be critical for the neuronal deterioration associated with Alzheimer’s disease (AD).3,4 These findings have led to a proposal that Alzheimer’s disease is caused by a deficit of these neurotrophins or by a loss of their receptors in the brains of the patients.5,6 Consistent with this hypothesis, a reduction of gene expression of NGF receptor is found in the nucleus basalis of AD patients.7 In addition, analysis of postmortem samples of hippocampus found a profound decrease in both mRNA and protein levels of BDNF in AD patients as compared with normal individuals.6,8 Moreover, a topical application of NGF into the brain of the patients exhibited a significant reduction of the symptoms, including dementia.9 However, the clinical utility of neurotrophin injection is limited by the need for invasive neurosurgical procedures. We have shown that L-tryptophan (L-Trp) and its metabolites, especially L-kynurenine, profoundly stimulated accumulation of mRNA and protein for NGF in the primary culture of mouse astrocytes.9,10 Systemically administered L-Trp enters the brain and is metabolized there via the kynurenine pathway, since i.p. injection of radiolabeled L-Trp causes production of labeled metabolites in the brain such as anthranilic acid, 3-hydroxyanthranilic acid, xanthene acid, and kynurenine acid.11 Addition of L-Trp to a diet leads to a significant increase in the brain levels of L-Trp and its metabolites, serotonin and 5-hydroxyindoleacetic acid.12 In view of these findings, we evaluated, in this study, the effects of addition of L-Trp to a tryptophan-deficient diet on the levels of NGF, BDNF, and NT-3 in various regions of the brain. The results demonstrate that the mice fed on a L-Trp-deficient diet had decreased NGF immunoreactivity in the hippocampus and reduced BDNF protein in the cerebral cortex.

Materials and Methods

Animals and diets. Male mice of the ddY strain were purchased from Shizuoka Experimental Animals Co-Operatives, Hamamatsu, and used at 2 to 3 months of age. The following animal experiments were done according to the guidelines for animal experimentation in our university. The mice were divided in three groups, each containing 5–6 animals. The mice were given free access to a Trp-deficient (0.1%, in total), adequate (0.25%), and excess (1.25%) Trp diets (Table 1), respectively, for 2 wk.

Measurement of neurotrophins. Various regions of the brain were dissected, weighed, and frozen on ethanol-dry ice and stored at −80°C until assay. For measurement of BDNF, dissected tissues were homogenized with 10 volumes of 100 mM sodium phosphate buffer (pH 7.0), containing 1 mM EDTA, 2 mM guanidine-hydrochloride (pH 7.2), and three protease inhibitors, 10 mM N-ethylmaleimide, 0.36 mM pepstatin, and 1 mM phenylmethylsulfonyl fluoride (Nacalai Tesque, Kyoto). Homogenates were sonicated and centrifuged at 46,000 g for 30 min at 4°C. BDNF in the super-
Table 1. Composition of Diets

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Trp&lt;sup&gt;1&lt;/sup&gt;-deficient diet</th>
<th>Adequate Trp diet</th>
<th>Excess Trp diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat gluten&lt;sup&gt;2&lt;/sup&gt;</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Corn starch</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Sucrose</td>
<td>500</td>
<td>499</td>
<td>490</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Corn oil</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Minerals&lt;sup&gt;3&lt;/sup&gt;</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Vitamins&lt;sup&gt;3&lt;/sup&gt;</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Choline-bitartrate</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td>0</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>(L-Trp in total)&lt;sup&gt;3&lt;/sup&gt;</td>
<td>(1.5)</td>
<td>(2.5)</td>
<td>(12.5)</td>
</tr>
</tbody>
</table>

1 L-tryptophan.
2 Wheat gluten contains L-Trp at a concentration of 780 mg/100 g.
3 \( \beta \)-d-galactosidase-labelled Fab' fragments prepared from pAbC9 and incubated at 4°C for 16 h.

Feeding the mica a Trp-deficient diet for 2 wk significantly lowered NGF in the hippocampus, while there was no difference in the the level of BDNF nor NT-3 in the region from those fed an adequate Trp diet (Fig. 1). Feeding an excess Trp diet produced no further change in the hippocampal levels of any of these neutrophins over that in the mice fed on an adequate Trp diet. The content of BDNF in the cerebral cortex decreased significantly when the mice were fed a Trp-deficient diet for 2 wk, while the levels of NGF and NT-3 were not modulated upon feeding of the diet. The dietary Trp level had

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Statistical analysis. Student's t-test was used to evaluate the significance of the data in each experiment.\textsuperscript{10}

Results
Feeding the mice a Trp-deficient diet for 2 wk significantly lowered NGF in the hippocampus, while there was no difference in the the level of BDNF nor NT-3 in the region from those fed an adequate Trp diet (Fig. 1). Feeding an excess Trp diet produced no further change in the hippocampal levels of any of these neutrophins over that in the mice fed on an adequate Trp diet. The content of BDNF in the cerebral cortex decreased significantly when the mice were fed a Trp-deficient diet for 2 wk, while the levels of NGF and NT-3 were not modulated upon feeding of the diet. The dietary Trp level had

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Fig. 1. The Effects of the Dietary Levels of L-Trp on the Concentration of NGF (a), BDNF (b) and NT-3 (c) in Various Brain Regions. The values are the mean±SEM for 5-6 animals \( *P<0.01 \) as compared with the animals fed on a Trp-deficient diet.
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no significant effect on the content of NGF, BDNF, or NT-3 in the entorhinal cortex nor in the septum of the mice.

Discussion

The results of this study demonstrate that the mice fed on a Trp-deficient diet had a significant decrease in NGF immunoreactivity in the hippocampus and a reduction of BDNF protein content in the cerebral cortex, while no meaningful change was observed with the brain levels of NT-3.

These findings indicate that the levels of these neurotrophins are regulated differentially with regard to their molecular species and to the discrete regions in the brain, depending on the dietary levels of Trp. Although this diversity may be physiologically meaningful, although its reason can not be explained properly now.

It was also shown here that feeding a Trp-excess diet produced no further increase in the brain levels of any of these neurotrophins. These results seem to contradict the results of our previous in vitro studies; tryptophan and its metabolites, especially kynurenine, significantly increased the synthesis of both NGF and BDNF in the primary culture of the mouse astroglial cells.1,10 These results suggest the presence of some regulatory mechanisms in the brain for preventing the brain levels of these neurotrophins from exceeding the normal ones.

Neurotrophins such as NGF, BDNF, and NT-3 are generally believed to be synthesized and released from neuronal cells in response to excitatory and inhibitory neuronal activity or to neurotrophins by themselves.17,18 Recently, there has accumulated evidence indicating that neurotrophins are also synthesized in non-neuronal cells such as glial cells and fibroblasts.19-23 The results of our previous findings strengthen the earlier evidence; L-Trp and its metabolites, especially L-kynurenine, significantly stimulate production of NGF in the primary culture of mouse astrocytes.9,10 Indeed, the enzymes associated with the kynurenine pathway in the brain are localized in glial cells.24 Nevertheless, it remains uncertain, even from the results of this study, whether modulation of neurotrophin production by dietary L-Trp occurs in neuronal cells or in glial cells in the brain.

L-Trp is a most limiting amino acid in a variety of foods, especially cereals and vegetables, and, hence, human beings become easily deficient in the amino acid. Therefore, the results of this study may imply an important etiological meaning in the pathogenesis of diseases. Since L-Trp is a natural food constituent, its toxicity, if any, may be least even when it is administered per os to patients at relatively large doses and for a long time. Therefore, it will be an useful method for treating the diseases.

References

