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Source: Zoological Science, 20(2) : 121-124

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.20.121

ZOOLOGICAL SCIENCE **20**: 121–124 (2003)

[SHORT COMMUNICATION]

Effect of Acute L-Tryptophan Exposure on the Brain Serotonergic System and Behavior in the Male Medaka

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ABSTRACT—The aim of this study was to investigate the effect of exposure to L-tryptophan (TRP) on the metabolism of 5-hydroxytryptamine (5HT) and behavior of medaka. In the first experiment, the fish were exposed to a 0, 1, 2 or 4 g/l of TRP solution for 24 hr. Although no significant difference in the brain 5HT content was detected, 5-hydroxyindoleacetic acid (5HIAA), a major 5HT metabolite, increased in a dose-dependent fashion. In the second experiment, the fish were maintained in a 0 or 4 g/l of TRP solution for 28 hr, and then their behaviors were monitored. The fish reared in under TRP solution were divided into two groups and transferred to either fresh water or a TRP solution. The locomotion of the TRP-treated group significantly increased compared to the control group irrespective of water conditions. It was suggested that TRP exposure activated the brain 5HTnergic systems and stimulated behavior of medaka.

Key words: behavior, 5-hydroxyindoleacetic acid, 5-hydroxytryptamine, L-tryptophan, medaka

INTRODUCTION

L-tryptophan (TRP) is the precursor of 5-hydroxytryptamine (5HT). 5HT is one of the neurotransmitters in the central nervous system, and the synthetic pathway of 5HT seems to be highly conserved across vertebrates (Parent, 1981). The neuronal events involving brain 5HT have been described in multiple aspects of physiology and behavior in various vertebrates, ranging from teleost fish to primates (Larson and Summers, 2001). In particular, brain 5HTnergic systems are involved in feeding, aggression, depression and stress behavior (Larson and Summers, 2001).

In rainbow trout, addition of TRP to the diet increased the brain 5HT metabolism and suppressed aggressive behavior (Winberg *et al*., 2001). Since 5HT was reported to suppress aggressive behavior in teleost fish (Adams *et al*., 1996), it was suggested that TRP stimulated brain 5HTnergic systems and then altered aggressive behavior. This result demonstrated that exogenous TRP can modify the brain 5HTnergic activity. The brain 5HT is believed to be involved in locomotion activity in mammals (Dringenberg *et al*., 1995; Lipska *et al*., 1992) and fish (Genot *et al*., 1984). To the authors' knowledge, however, no information was available for the effect of TRP on locomotion in fish.

Medaka (*Cyprinodontiformes Oryzias*) is commonly used for behavioral studies (Iwamatsu, 1997). The aim of the present study was to investigate the metabolism of 5HT in the brain following exposure of the fish to several concentrations of TRP. In addition, the behavior of the fish was monitored after rearing in a TRP solution.

MATERIALS AND METHODS

Fish

Male medaka (FLFII strain, approximate body weight 450 mg) were reared by the Laboratory of Fisheries Environmental Science in Kyushu University. All the fish were maintained in a tank with distilled water at 25°C under a 16 hr light and 8 h dark (lighting on 5:00–21:00) photoperiod for a day before the start of the experiment. They were given a diet (*Artemie nauplii*) twice daily.

TRP solution

TRP provided from Kyowa Hakko Kogyo (Tokyo, Japan) was dissolved in distilled water. The control group was exposed to distilled water. In all experiment, each solution was kept at 25℃.

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Experiment 1: Effect of TRP exposure on the brain 5HT level in medaka

Experiment 1 was carried out to examine the effect of TRP exposure on the brain 5HT level in medaka (7 to 8-month-old). The fish were placed in a tank with 0 (control), 1, 2 or 4 g/l of TRP solution for 24 hr and five fish were reared in each tank. They were then decapitated and their brains quickly removed, weighed, quickly frozen by dry ice, and stored in a freezer at –85°C.

The concentrations of 5HT and 5-hydoroxyindoleacetic acid (5HIAA), a major metabolite of 5HT, were determined using high-performance liquid chromatography (HPLC) with an electrochemical detector. The brain samples were homogenized and deproteinized using 200 µl of 0.2 M ice-cold perchloric acid containing 100 µM Na_2-EDTA and 2.4 ng/ 30 µl isoproterenol as an internal standard. The homogenate was centrifuged at 10,000 x g and 4°C for 4 min. Then the supernatant was filtered with a centrifuge-filtration unit (Ultra Free C3-GV, Millipore, Bendford, MA, U.S.A) at 10,000 x g and 4°C for 4 min. The 30 µl of filtrate was injected into a HPLC system (Eicom, Kyoto, Japan) with a 150×2.1 mm ODS column (SC-50DS, Eicom, Kyoto, Japan). The mobile phase was 0.1 M sodium acetate- 0.1 M citric acid buffer (pH 3.5) containing 2.3 mM sodium 1-octane sulfonate, 1.0 mM disodium ethylenediaminetetraacetic acid and 17% methanol. 5HT and 5HIAA were detected using an electrochemical detector (Model ECD-300, Eicom, Kyoto, Japan) at an applied potential of +0.70 V. The external standard was used to identify peaks eluting in the chromatogram according to retention time and conformation. The detection limit of the system was 0.1 pg/sample.

Experiment 2: Effect of TRP exposure on locomotion activity of medaka

Experiment 2 was carried out to investigate the effect of TRP exposure on locomotion activity of medaka (8 to 9-month-old). The fish were maintained in a group before the experiment. They were divided into three groups and then were exposed to a 0 (control) or 4 g/l of TRP solution for 28 hr. After that, individuals were transferred into a novel environment, a white circular basin (diameter 24 cm) containing 500 ml of a 0 or 4 g/l TRP solution (water depth 3 cm) to determine locomotion activity. To consider osmotic effect, TRP group was divided into two groups. Namely, we prepared three groups (five fish per group): 1) maintained in distilled water (none of TRP) and their behavior monitored in water (Water-Water), 2) exposed to a TRP solution and then their behavior monitored in water (TRP-Water) and 3) exposed to a TRP solution and their behavior monitored while in a TRP solution (TRP-TRP).

Locomotion activity was determined with a video image motion analyzer (AXIS-90, Neuroscience, Inc. Japan). The image of individual fish was captured with a CCD camera which was connected to the analyzer. The locomotion activity of the fish was recorded every minute for 5 min and was expressed as the cumulative moved distance.

Statistical analysis

Statistical analysis was made using a commercially available package, StatView (Version 5, SAS Institute, Cary, U.S.A., 1998). Data were analyzed by one-way analysis of variance (ANOVA), and Fisher's protected least square difference (PLSD) test as a post hoc test was used in Experiment 1. Furthermore, regression equations were applied. Data in Experiment 2 were analyzed by two-way repeated measures ANOVA. The results are presented as means ±SEM.

RESULTS

Experiment 1: Effect of TRP exposure on the brain 5HT level in medaka

Fig. 1 shows the effect of TRP exposure on 5HT and its metabolite in the medaka brain. The concentration of 5HT was not affected by any TRP treatment. On the other hand, TRP significantly increased the concentration of 5HIAA in a dose-dependent manner. The effect of TRP exposure on

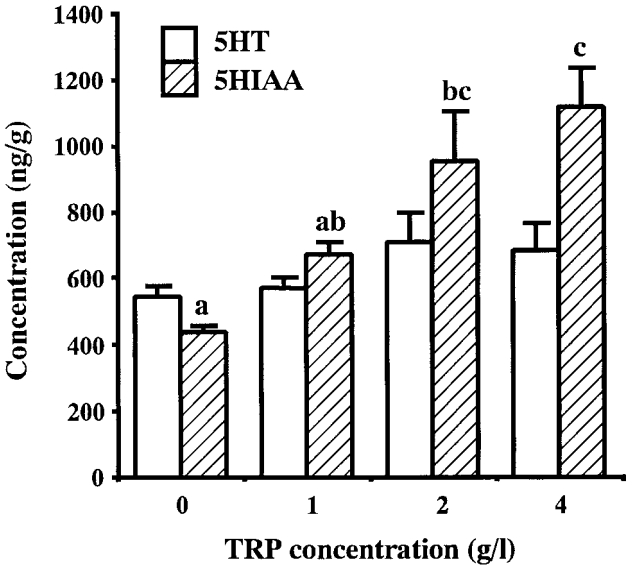

Fig. 1. The brain concentrations of 5HT and 5HIAA in medaka exposed to TRP solution (0, 1, 2 or 4 g/l) for 24 hr. Data are expressed as means ± SEM. The number of fish used was five each. Groups with different letters are statistically different (P<0.05). When regression was fitted, the following equations were obtained; concentration of 5HIAA (ng/g) = 501.7 (S.E. 77.1)+ 168.2 (S.E. 33.6) TRP (R^2 = 0.581, P<0.0001).

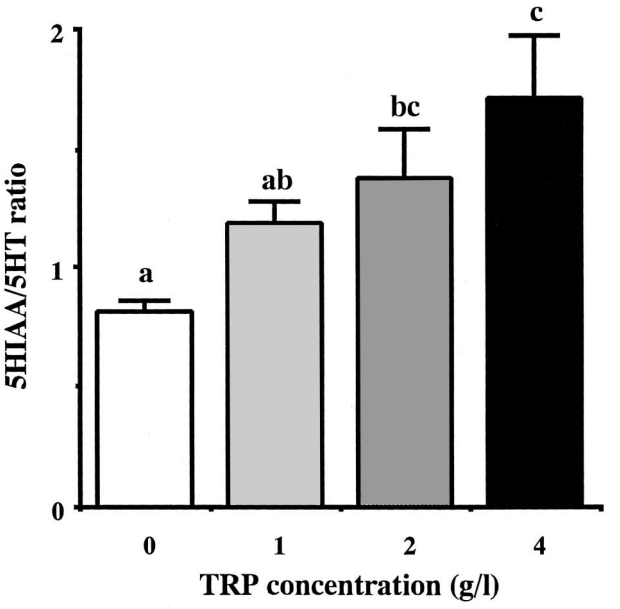

Fig. 2. The brain 5HIAA/5HT ratio in medaka exposed to L-tryptophan solution (0, 1, 2 or 4 g/l) for 24 hr. Data are expressed as means ± SEM. The number of fish used was five each. Groups with different letters are statistically different (P<0.05). When regression was fitted, the following equations were obtained; 5HIAA/5HT = 0.901 (S.E. 0.129)+ 0.214 (S.E. 0.056) TRP (R^2 = 0.443, P<0.001).

5HIAA/5HT, used as the index of 5HT turnover ratio in brain, is shown in Fig. 2. 5HIAA/5HT significantly increased in a dose-dependent manner.

Experiment 2: Effect of TRP exposure on locomotion activity of medaka

The effect of exposure to TRP on the cumulative moved distance is shown in Fig. 3. The cumulative moved distance in the TRP treated groups (TRP-Water and TRP-TRP) was significantly greater than that in the Water-Water group. A significant time effect was observed. A significant interaction between TRP treatment and time was detected.

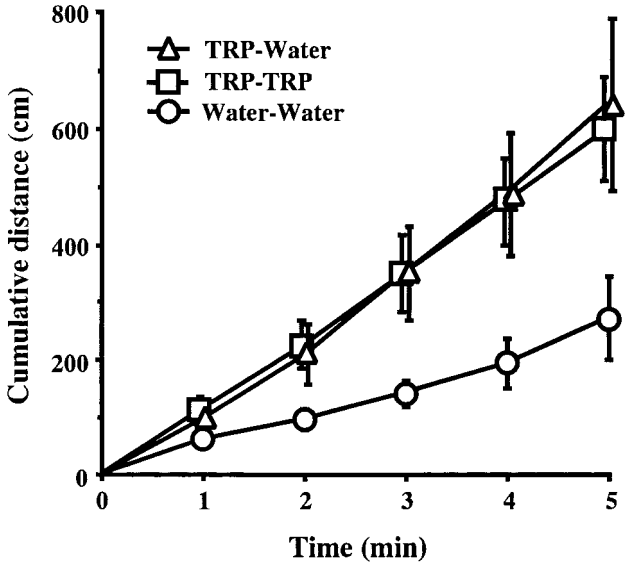

Fig. 3. The cumulative distance of locomotion in novel environment with 0 or 4 g/l of TRP solution. Data are expressed as means ± SEM. The number of fish used was five each.

DISCUSSION

The present study revealed that the exposure to a TRP solution increased brain 5HIAA concentration, but not 5HT concentration, resulting in the increase in 5HIAA/5HT in medaka. This result was similar to the report by Winberg *et al*. (2001) that high dose of dietary TRP increased the brain 5HIAA concentration and 5HIAA/5HT ratio in rainbow trout. In the present study, TRP was added to an environmental water, but not to a diet. In this case, it was considered that TRP entered into the body of medaka through both oral and biomembrane routes, since amino acid can pass biomembrane (Iwata and Hirano, 1991). It was reported that exogenous TRP enhanced rates of 5HT synthesis and release in rats (Boadle-Biber, 1993). It was possible that 5HT release was increased by the TRP exposure in the present study, since the ratio of 5HIAA/5HT increased. Shannon *et al*. (1986) indicated that the electrical stimulation of brain 5HTnergic neurons elevated the concentration of 5HIAA and 5HIAA/5HT while 5HT concentration was unchanged. Thus, TRP exposure might stimulate the brain 5HTnergic systems in medaka without changes in 5HT concentration. It was also suggested that the TRP exposure stimulated 5HT synthesis in the present study, since Aldegunde *et al*. (2000) demonstrated TRP hydroxylase, a rate-limiting enzyme for 5HT synthesis, to be activated by exogenous TRP in rainbow trout.

The locomotion in a novel environment was significantly increased by exposure to TRP in the present study. Because locomotion activities of both TRP groups were similar, it was indicated that osmotic difference did not affect the locomotion activity at least in the present study and was suggested that the increase in the locomotion activities was induced by TRP exposure itself. Genot *et al*. (1984) demonstrated inhibition of 5HT synthesis reduced the spontaneous locomotion activity and administration of 5-hydroxytryptophan, a precursor of 5HT, restored the activity in eels. This and the present results suggested that the increase in locomotion activity might be due to the activation of the brain 5HTnergic system induced by the TRP exposure.

It was reported that an intracerebroventricular injection of corticotrophin-releasing hormone (CRH) increased locomotion in an unfamiliar environment in salmon (Clements *et al*., 2002). CRH activates hypothalamus-pituitary-adrenal axis (HPA axis) and then stimulates glucocorticoid, including cortisol, in mammals (Herman and Cullinan, 1997). In teleost fish, the hypothalamus-pituitary-interrenal axis (HPI axis) is reported to function similar to the mammalian HPA axis (Winberg *et al*., 1997). Winberg *et al*. (1997) reported that plasma cortisol level was increased by administration of a 5HT receptor agonist, indicating that 5HT stimulates CRH neurons. Therefore, it is possible that activation of the 5HTnergic system by TRP exposure stimulated CRH neurons, and then increased locomotion activity in the present study. However, no information was obtained about HPI axis and CRH neurons in medaka although corticotroph was found (Iwamatsu, 1997). The information of CRH neurons in the madaka brain will be needed to clarify how TRP exposure stimulated locomotion activity in medaka.

The present study suggested that TRP exposure activated the brain 5HTnergic system and then facilitated the locomotion activity in medaka.

ACKNOWLEDGEMENTS

This study was supported by grant-in-aid for scientific research from the Ministry of Education, Science and Culture, Japan. We are grateful to Dr. D.M. Denbow, Virginia Polytechnic Institute and State University, USA, for his reading of the manuscript.

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(Received September 2, 2002 / Accepted November 5, 2002)