Assessment of the Dose-Dependent Effect of Tryptophan on Anxiety with Electrodermal Activity and Elevated Plus Maze Test in Mice*

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INTRODUCTION

Serotonin is a monoaminergic neurotransmitter involved in a wide variety of brain functions such as mood control, the regulation of sleep and body temperature, anxiety, drug abuse, food intake, and sexual behavior (1). As an essential amino acid, L-Tryptophan (L-TRP) is the precursor of the neurotransmitter 5-hydroxy-tryptamine (serotonin, 5-HT) (2). The effects of TRP are often attributed to its conversion in the CNS to 5-HT (3), which is well known to be associated with anxiety (4).

The putative role of 5-HT in several neuropsychological functions is still a matter of debate. According to classic theory, the function of 5-HT is related to the level of anxiety (5). There are many studies to assess the role of the...
serotonergic system in various aspects of human behavior.

The method of nutritional TRP depletion (TD) has been frequently used as a tool to reduce systemic TRP levels (6). TD has recently been used to investigate the involvement of serotonergic mechanisms in anxiety disorders (7).

Administration of TRP in humans caused an anxiolytic effect (8), and depressed patients showing primarily anxiety and/or aggression reportedly responded to TRP therapy (9). However, there is lack of conclusive evidence from clinical trials for clinical effectiveness against anxiety.

Intravenous (i.v.) infusion of TRP has been used extensively in the past decade as a pharmacologic probe of 5-HT function in depressed patients, both before and during antidepressant treatment (10). There are a few studies to assess the effect of per os (p.o.) or intraperitoneally (i.p.) administration of TRP on anxiety or other diseases (11).

In present study, we aimed effects of i.p. L-TRP on animal’s anxiety with electrodermal activity (EDA). EDA is an important method used in studies of psychophysiological behavior. The attention and perception centers of the central nervous system play an important role in the formation of EDA. It is recorded as a change in skin potential or resistance of sweat gland reaction controlled by the sympathetic nervous system (12). The relationship between anxiety and EDA has been widely studied. The results of the studies on human subjects also indicate that anxious subjects have greater sweat gland activity [shown by skin conductance level (SCL)] than nonanxious or low anxious subjects (13). However, data in animals are limited.

Additionally, in present study, the elevated plus-maze test was used to measure unconditioned anxiety-related behavior. The elevated plus-maze test, a well-established animal model of anxiety, is based upon the natural aversion of rodents to heights and open space and has been validated for both rats (14) and mice (15). Certain pharmacological compounds can have dose-dependent treatment effects on anxiety-related behavior (16). Dose-related acute effects of single dose L-TRP on anxiety have not been well investigated. The present study was undertaken: a) to determine the L-TRP’s dose-related effects on anxiety on the elevated plus-maze test b) to determine the relationship between the behavioral scores of mice tested on the elevated plus-maze test and EDA.

METHODS

Animals. 45 male Swiss mice (10–12 weeks) were housed in groups of 3–6 / cage (60 _ 60 _ 30 cm polypropylene cages) under a reversed 12-h light-dark cycle (lights on at 07.00 hours) and in a constant temperature and humidity controlled environment (22±2°C and 50%±5, respectively). The animals had a period of adaptation for three days and were allowed free access to food and water, prior the experiments. Each animal was used once. All experiments were performed according to the guidelines of the Erciyes University Ethics Committee for the welfare of experimental animals (no.02-011-4) and complied with the guidelines for animal experimentation of the National Institutes of Health (17).

Treatment Schedules and Drugs. Physiological saline was injected with equal doses to the control group (n=15). The following L-TRP doses were used; L-TRP 250 mg/kg (Group 1, n=15), L-TRP 500 mg/kg (Group 2, n=15). L-TRP was obtained from Sigma (USA) and freshly prepared in saline and given i.p. as single dose 30 min before testing.

Elevated plus-maze. Anxiety-related behavior was measured by the elevated plus-maze test. The elevated plus-maze was a modification of that validated for Swiss mice by Lister (18). It was made of Plexiglas. It consisted of two open arms and two enclosed arms (30 cm long and 5 cm wide) that were opposite each other. The arms extended from a central plate, which is also made of Plexiglas (5 cm long and 5 cm wide). The wall of the enclosed arms was 15 cm in height and 1 cm in thickness. The plus-maze stood on a stand, so that it could be elevated approximately 40 cm above the ground. The central platform and maze floor were made from opaque Plexiglas while the walls of the closed arms were transparent Plexiglas (19).

Skin conductance. SC was recorded between the paw pads of both hindlimbs using Ag/AgCl electrodes after the maze test. NaCl electrode (0.05 M) jelly was
placed between the skin and the electrodes. Electrodes were connected to a SC unit that was built in our laboratory. The technical specifications of this unit have been published elsewhere (20). Briefly, it conforms basically to the Lykken-Venables method (21). SC unit output was digitized and stored on-line by an IBM-AT computer. The mean of SC recorded for 2 min was expressed as SCL [ln (µmho)/cm² per electrode area].

Procedure. Experiments were started with 15 mice in each group. The mice received daily i.p. injections of L-TRP or physiological saline. The elevated plus maze tests started 30 min after the injections. Each mouse was individually placed on the center of the platform of the maze facing an open arm. The mouse was allowed a 5-min pretest habituation period to explore the maze (22). Following this period, three measures were taken during test period: 1) total arm entries (by all four paws); 2) entries into the open arms; 3) total time spent in open arms. The mouse was removed following a 5-min test session. The time spent in the open arm and the number of enter were calculated by a PC computer. As soon as the mouse entered one of the open arms, experimenter pressed a key. When the front paws of the mouse have crossed into central zone the experimenter pressed another key. The time (in seconds) between pressings the keys and the number of entries were calculated. The same procedure for closed arms was repeated with the other two keys. Because time spent on the open arms and entries the open arms are the best measure of general anxiety in the maze. Open arm entries were expressed as percentage of total entries. The elevated plus maze test apparatus was wiped clean with a daub sponge and dry cloths between tests. Behavioral testing was always carried out between 09.00 and 15.00 hours. After elevated plus maze test, SCL was recorded, without losing any time.

Statistical Analysis

All data are presented as means ± standard deviations (SD). The data were analyzed using one-way analysis of variance (ANOVA) followed by the post-hoc Scheffe test. Statistical analysis was performed using a software package (SPSS 10.1). The level of significance was defined as p < 0.05.

RESULTS

Effect of L-TRP on Anxiety of Mice in the Elevated Plus-Maze.

Table 1 indicates the mean time spends and entries (%) in the open arms. There were statistically significant differences between groups at the mean time spend in the open arms [F(2,42)=3.405; p=0.043]. According to Posthoc Scheffe test, there was statistically significant difference between Group 1 and Group 2 (p<0.05). Group 1 had got lower mean time spend in the open arms than Group 2. This group also had got lower mean time spend in the open arms than control group, but it wasn’t statistically difference. The mean time spend in the open arms was higher in Group 2 than control group. But it wasn’t statistically difference. There was no statistically difference among groups about entries (%) in the open arms. Given 30 min before testing, while Group 1 exerted anxiogenic effect, Group 2 exhibited anxiolytic effect in the elevated plus maze.

### Table 1: Skin conductance levels and elevated plus-maze test scores in mice with different L-TRP doses

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>SCL (µmho)</th>
<th>Time spent in open arms (s)</th>
<th>Percentage of time spent in open arms (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>15</td>
<td>1.22±0.19</td>
<td>22.6±24.42</td>
<td>7.33±10.23</td>
</tr>
<tr>
<td>Group 1</td>
<td>15</td>
<td>1.39±0.15*</td>
<td>6.40±7.4</td>
<td>4.60±6.86</td>
</tr>
<tr>
<td>Group 2</td>
<td>15</td>
<td>1.17±0.17*</td>
<td>41.2±58.06*</td>
<td>3.00±1.06</td>
</tr>
<tr>
<td>All mice tested</td>
<td>45</td>
<td>1.26±0.19</td>
<td>23.42±38.56</td>
<td>4.97±7.2</td>
</tr>
<tr>
<td>ANOVA (p value)</td>
<td></td>
<td>0.044</td>
<td>0.043</td>
<td>0.255</td>
</tr>
</tbody>
</table>

The data are means ±SD. SCL: skin conductance level; Group 1: L-TRP 250 mg/kg; Group 2: L-TRP 500 mg/kg; *: significantly different when compared with control group (Scheffe test); #: significantly different when compared with group 1 (Scheffe test).
Effect of L-TRP on skin conductance level

Table 1 indicates the mean SCL values in all groups. There were statistically differences between groups [F(2,42)= 6.442; p<0.05]. According to Posthoc Scheffe test, in the Group 1, SCL was higher than control group’s (p<0.05). Group 1 exerted anxiogenic effect than control group. In Group 2, there was no statistically significant difference with control group. But SCL was lower in Group 2 than control group.

There was also statistically significant difference between Group 1 and Group 2. Group 2 had the lowest SCL among the groups. Group 2 exerted anxiolytic effect with this dose (p<0.05).

DISCUSSION

We have investigated the anxiogenic effects of L-TRP in mice using elevated plus-maze test and electrodermal activity recording. In this study, the mice, which were given high dose L-TRP exhibited increased anxiolytic effect and in the low dose L-TRP group, we found anxiogenic effect using elevated plus-maze test and electrodermal activity recording.

The method of manipulating the serotonergic system (e.g. acute or chronic) or the use of specific types of 5-HT modulating agents (receptor-specific affinities) can generate different behavioral effects (5).

The involvement of 5-HT in anxiety is quite controversial. On the one hand, activation of the serotonergic system by 5-HT precursors, agonists or reuptake inhibitors has been described as anxiogenic both in animal models (23) and in humans (24). The exposure of rats to a novel aversive environment increases the release of 5-HT in the frontal cortex and hippocampus (25), while the administration of a 5-HT receptor agonist, m-chlorophenyl-piperazine (mCPP), induces anxiety both in humans and in animal models (24). Conversely, treatment with 5-HT2 and 5-HT3 receptor antagonists apparently reduces anxiety in rodents (23) On the other hand; agonists of the 5-HT1A autoreceptors have proven to be anxiolytic (26).

Certain pharmacological compounds can have dose-dependent treatment effects on anxiety-related behavior (27). However, it is not clear whether the action of 5-HT in animal models truly represents anxiogenesis or anxiolytic effect examined the exploratory behaviors of rats in the plus-maze as dose dependent. In the literature, dose range tried only up to 250 mg/kg. So, we want to evaluate L-TRP’s anxiolytic or anxiolytic effects higher than 250 mg/kg.

In present study elevated plus maze test was used to measure unconditioned anxiety-related behavior. We found that in the low dose L-TRP group (250 mg/kg), mice made more frequent entries into closed arms and stayed a longer time compared to given high dose L-TRP (500 mg/kg) and control group. Thus, low dose L-TRP group exerted anxiogenic effect; high dose L-TRP group exhibited anxiolytic effect in the elevated plus maze.

According to Wong and Ong study, single-dose TRP caused an antidepressant-like effect dose dependently up to 125 mg/kg. When the TRP dose was increased to 250 mg/kg, i.e. a reversal of effect occurred at high dose. An antianxiety-like effect was observed for TRP only at 250 mg/kg dose together with p-chlorophenylalanine pretreatment in mice using the forced-swimming test, open-field test (9). These findings are consistent with our results.

Alternatively, it has been reported that doses of tryptophan (0, 12.5, 50, 75, 100, 125 and 200 mg/kg) have antidepressant-like properties in Porsolt’s swim test (11). They found that TRP exhibited a U-shaped dose response relationship in the forced-swimming test and the effective dose range was reported was only up to 75 mg/kg as opposed to observed 125 mg/kg in the Wong and Ong studies (9).

The biphasic effect of L-TRP on anxiety could be a possible explanation that excess L-TRP may be converted not into 5-HT but into secondary metabolites of the kynurenine pathway (28). It has been proposed indeed that acute L-TRP administration decreases the firing rate of 5-HT neurons (29), reflecting an activation of 5-HT1A autoreceptors (30). In any case, as suggested above, the production of metabolites from the kynurenic pathway may explain some discrepancies between TRP availability, 5-HT activity and the expected behavioral responses. The kynurenines are mainly represented by quinolinic and kynurenic acids with antagonistic properties, at least regarding neuroprotection, that may explain the complexity of their described effects in anxiety and depression (31).
Assessment of the dose-dependent effect of tryptophan on anxiety with electrodermal activity and elevated plus maze test in mice

Comparing the aggregated scores of the ‘plus maze’ with parameters of other models of anxiety, such as the ‘open field test’ and the ‘light–dark box’, resulted in high correlation scores and moreover, similar treatment effects were found in these different tests (7, 32) consistent with our Group 1 results. However, Lieben et al. found that TRP depletion did not affect anxiety-related behavior as tested in the open field (i.e. occupation time and distance moved) (7).

To explain the biphasic effect of L-TRP concerning the 5-HT function on anxiety, we must take into account other factors. Results can depend on the type of strain (33) or on age-related variability (34). Besides this, the type of test used for measuring anxiety often reflects different traits (e.g. exploratory behavior, conflict situation (35).

A recent study (36) suggested the utility of electrodermal activity parameter, as a tool to assess anxiolytic and anxiogenic activities of some drugs. Our electrodermal findings indicated that, in the low dose L-TRP group, mice had the highest SCL. High SCL values showed us increased anxiety-like behavior. These results are consistent with elevated plus maze findings. SCL values decreased when the dose was increased in Group 2.

In the literature, there is no study about L-TRP and EDA. But we know that the autonomic nervous system appears to be involved in the stress-related changes in brain tryptophan, and this effect is due to the sympathetic rather than the parasympathetic limb of the system. The activation of the sympathetic nervous system is responsible for the stress-related increases in brain tryptophan, probably by enabling increased brain tryptophan uptake (37). Since muscarinic acetylcholine receptors in the sweat glands stimulate sweat secretion, and tryptophan may affect muscarinic properties, the higher SCL in tryptophan administration could be due to a peripheral pharmacological action (38). Similar mechanisms, which explaining in the elevated plus maze results may be responsible from low SCL with administration of high dose L-TRP.

Conclusions

Results of the present work confirm that L-TRP produces a dose-dependent effect in the mice. L-TRP results in anxiogenic behavior based on plus maze and EDA at 250 mg/kg i.p. At high dose of L-TRP, this anxiogenic effect has been disappearance. The anxiety-like effect of L-TRP depends on its dose-related effect. However, this is a preliminary study and future evaluations are certainly needed to confirm these anxiolytic or anxiogenic effects in different L-Tryptophan doses.

Kaynaklar:

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