Effects of Soybean Food Pellets on m-CPP-Induced Anxiety Model of Mice

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In this study, we gave the soybean powder-added food pellets (soybean pellets) to investigate anti-anxious effects of soybean in male mice. Twenty eight days after feeding control pellets or soybean pellets, we observed the behavioral changes in the elevated plus maze. There was no significant difference on the time spent in the open arms (%) between mice fed the control and soybean pellets. When we administered m-chlorophenylpiperazine (m-CPP, 2.5 mg/kg, i.p.) to mice, the mice fed control pellets showed the decrease in the time spent in the open arms, suggesting that anxiety-like behavior was induced by m-CPP. On the other hand, we could not observe the m-CPP-induced anxiety-like behavior in mice fed soybean pellets in this test. These results suggest that soybean pellets may attenuate anxiety-like behavior in mice.

Key words soybean; anti-anxious effect; elevated plus maze; animal model

In Japan, soybean is one of the most important foods and the Japanese have eaten since ancient times. It contains several biologically active components that may contribute individually or synergistically to the health benefit. As consumers in the world are becoming increasingly aware of the health benefits of soybean, attention has turned to the way to take soybeans that provide improved taste, functional characteristics, and health benefits.

Many studies in the biological field have demonstrated the effect of soy isoflavones on signaling pathways, including but not limited to, cell proliferation and differentiation, cell cycle regulation, apoptosis, angiogenesis, cell adhesion and migration, metastasis, and activity of different enzymes in vitro. Experimental and epidemiological evidence in the life science suggest that soybean-derived products can prevent some diseases such as cancer, cardiovascular disease and osteoporosis. Soybean also contains soy protein, isoflavones, fiber and saponins, and recent works revealed that isoflavones have significant effects on some diseases of animal models.

It is not well known the usefulness of soybean itself as a food on the brain function, whereas it has been clarified that soy-extracted phytoestrogens (isoflavones) may regulate the conventional parameters consisted of the frequency of open arms, closed arms and total arm entries, and the amount of time spent by mice in the open arms, on the central platform and in the closed arms of the maze. These data were also used to calculate open arm entries (%) [i.e. (open arm entries/total arm entries)×100] and time spent in the open arms (%) [i.e. (open time/open and closed time)×100]. m-Chlorophenylpiperazine, a serotonin receptor agonist (m-CPP; Sigma, U.S.A.) was dissolved in physiological saline, and administered intraperitoneally to mice 30 min before the

MATERIALS AND METHODS

Animals and Foods Five-week-old male ICR mice (Nihon SLC, Japan) were purchased. Animals were kept in a small home cage of controlled room (23 ± 1 °C, 50 ± 5% humidity) and were given access to food pellets and water ad libitum. The room lights were on between 7 h 30 and 19 h 30. We used control food pellet (AIN-93G; Oriental Yeast, Japan) and the ingredients are shown in the Table 1. Each of the food pellets contained standard nutrients and only a part of cornstarch was replaced with powdered soybean (Fukuyutaka). All experiments were performed in accordance with the Guidelines for Animal Experiments of Meijo University and the Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society (1987).

Elevated Plus Maze Test On the day 29 from the starting to feeding, we assigned mice to the elevated plus maze. The elevated plus-maze was consisted of two open (25×8×0.5 cm) and two closed arms (25×8×20 cm) emanating from a common central platform (8×8 cm) to form a plus shape. The entire apparatus was elevated to a height of 50 cm above floor level. Testing commenced by placing a mouse on the central platform of the maze facing an open arm, and a standard 5-min test duration was employed. Conventional parameters consisted of the frequency of open arms, closed arms and total arm entries, and the amount of time spent by mice in the open arms on the central platform and in the closed arms of the maze. These data were also used to calculate open arm entries (%) [i.e. (open arm entries/total arm entries)×100] and time spent in the open arms (%) [i.e. (open time/open and closed time)×100].

Table 1. Standard Ingredients in Food Pellet

<table>
<thead>
<tr>
<th>AIN-93G Constituents (%)</th>
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<tbody>
<tr>
<td>Cornstarch 39.7</td>
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<tr>
<td>Casein 20</td>
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<tr>
<td>L-Cystine 0.3</td>
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<tr>
<td>α-Cornstarch 13.2</td>
</tr>
<tr>
<td>Sucrose 10</td>
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<tr>
<td>Bean oil 7</td>
</tr>
<tr>
<td>Cellulose powder 5</td>
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<tr>
<td>Mineral 3.5</td>
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<tr>
<td>Vitamin 1</td>
</tr>
<tr>
<td>Choline bicitrate 0.25</td>
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<td>Butylhydroquinone 0.0014</td>
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Phytoestrogens in soybeans are proposed to have crucial effects on anxiety, and increased stress-related hormone in the plasma. Therefore we compared plasma corticosterone levels among four groups, but failed to detect significant differences (data not shown). It is possible that our soybean pellets might not affect the plasma corticosterone or might not have enough contents to change that hormone level.

In the elevated plus maze test, there were no remarkable differences between control pellets-fed mice and soybean pellets-fed mice on the time spent in the open arms. We observed the response when mice were treated with m-CPP, one of anxiogenic reagent, and then compared the effects of each food pellet on m-CPP-induced anxiety model. In mice fed control pellets, the time spent in the open arms of m-CPP treated mice was less than that of saline-treated mice in the closed arms, suggesting that m-CPP may induce anxiety as previous reports. To be noted, mice fed soybean pellets did not demonstrate the m-CPP-induced anxiety-like behavior. This m-CPP-induced anxiety model has been reported to mediate postsynaptic 5-HT₂c receptor but not 5-HT₂a and 5-HT₁b receptors. Taken our results together, soybean pellets are possible to modulate postsynaptic 5-HT₂c receptors, and we have to show the detail mechanism in the next steps. It is concluded that intake of soybean for a month may be potent on anxiety.

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