BACKWARD WALKING INDUCED BY L-5-HYDROXYTRYPTOPHAN IN MICE

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Abstract—An intraperitoneal administration of large doses of L-5-hydroxytryptophan (L-5HTP) induced dose-dependently a behavior characterized by backward walking in mice. D-5-hydroxytryptophan (D-5HTP) and L-3,4-dihydroxyphenylalanine (L-DOPA) in the same doses failed to induce such behavior. The backward walking induced by L-5HTP was blocked by a decarboxylase inhibitor, DL-α-methyl-DOPA, but was potentiated by a monoamine oxidase inhibitor (MAOI), tranylcypromine or pargyline. An intracerebral injection of L-5-hydroxytryptamine (5-HT) induced a similar backward walking which was potentiated by a MAOI. The backward walking induced by L-5HTP was completely inhibited by 5-HT and dopamine (DA) receptor blockers, but was not inhibited by α- or β-adrenergic blocker, antihistamine or anticholinergic drug. On the other hand, zimelidine and clomipramine, 5-HT uptake inhibitors, markedly potentiated the backward walking, while desipramine had no effect. From the results, it appears that excess amounts of 5-HT formed from L-5HTP produced the backward walking by directly stimulating the central 5-HT receptors, and DA is also involved in the behavior. This behavior may serve as a good model to assess the central serotonergic activity of drugs.

The administration of 5-hydroxytryptophan (5-HTP), some hallucinogens and benzodiazepines to mice can induce head-twitch (1-5). The head-twitch is reported to be related to the central action of 5-hydroxytryptamine (5-HT), because the incidence of this behavior was correlated with the increased amount of brain 5-HT after injection of 5-HTP (1, 4), and was decreased by antiserotonergic drugs, cyproheptadine and methysergide (1, 6). We observed that when large doses of L-5-hydroxytryptophan (L-5HTP) were given i.p. to mice, the animals showed a characteristic behavior of backward walking. It is likely that 5-HT, formed from L-5HTP in the central nervous system, plays an essential role in this behavior.

The present paper deals with the behavior of backward walking induced by L-5HTP, and with effects of neuroleptics and antidepressants on the behavior.

MATERIALS AND METHODS
Male ddY strain mice aged 4 weeks, weighing 16–26 g, were used.

Observation of behavior: Five mice at one time were placed on a table (45 cm × 70 cm). Backward walking was defined as moving backwards 10 cm or over. The presence or absence of the backward walking in each animal was examined for 1 min, respectively at 20, 30 and 40 min after dosing with L-5HTP. The percentage of
animals showing the backward walking was calculated at each observation time and the peak effects at either 20, 30 or 40 min after dosing with L-5HTP were used to express the results.

**Intracerebral injection:** Intracerebral injection was given according to the method of Haley and McCormick (7). The needle of a 100 μl microsyringe (MS100, Therumo) was inserted perpendicularly through the skull into the lateral ventricle of brain, and a fixed volume of 10 μl of 5-HT solution was injected. The site of injection was 2 mm lateral from midline to either side on a line drawn through the anterior base of the ears, and was 3 mm deep from the surface of head. The depth of the needle tip was adjusted with a guide cannula.

**Effects of drugs:** Effects of various drugs were investigated on the backward walking induced by L-5HTP. When inhibitory effects of drugs were examined, L-5HTP was given i.p. to mice in a dose of 500 mg/kg. On the other hand, when potentiating effects of test drugs on the backward walking were examined, L-5HTP was given i.p. to mice in a dose of 80 mg/kg. Test drugs were given i.p. by respective schedules as follows: DL-α-methyl-DOPA 1 hr, tranylcypromine 1 hr, pargyline 3 hr and other drugs 30 min before L-5HTP. Zimelidine, clomipramine and desipramine were given also by p.o. route with a stomach tube 60 min before L-5HTP. Control animals were given a vehicle instead of a test drug.

**Drug used:** L-5-hydroxytryptophan (L-5HTP) (Sigma), D-5-hydroxytryptophan (D-5HTP) (Sigma), L-3,4-dihydroxyphenylalanine (L-DOPA) (Nakarai Chemicals), DL-α-methyl-3,4-dihydroxyphenylalanine (DL-α-methyl DOPA) (Sigma), serotonin creatinine sulfate (Sigma), tranylcypromine hydrochloride (Sigma), pargyline hydrochloride (Sigma), zimelidine* (Astra), clomipramine hydrochloride (Fujisawa), desipramine hydrochloride (Fujisawa), fluphenazine dihydrochloride, chlorpromazine hydrochloride (Sigma), haloperidol (Janssen), cyproheptadine hydrochloride (Nihon Merck Banyu), methysergid bimaleate (Sandoz), diphenhydramine hydrochloride (Sigma), phentolamine mesylate (Regitine®, CIBA-Geigy), DL-propranolol hydrochloride (Sigma) and atropine sulfate (Merck). L-5HTP was suspended in 0.5% methylcellulose aqueous solution (0.5% MC). Haloperidol was dissolved in a minimum amount of 20% acetic acid and diluted with distilled water. Other drugs were dissolved in or diluted with saline.

*: Z-1-(4-bromophenyl)-1-(3-pyridyl)-3-dimethylaminopropen dihydrochloride hydrate (H102/09).

**Statistical analysis:** Calculations of p values were performed according to Fisher exact probability test.

**RESULTS**

**Dose-effect relationship in L-5HTP-induced backward walking:** After injection of L-5HTP to mice, the backward walking appeared at 10 min and almost disappeared at 60 min. Incidence of the backward walking (number of animals showing backward walking/number of animals used) peaked from 20 to 40 min with any dose of L-5HTP. Peak value of the incidence increased with increase in the dose of L-5HTP. As shown in Fig. 1, a linear positive correlation was observed between the peak values of the incidence and L-5HTP doses from 100 to 560 mg/kg. Nine of 10 animals showed the backward walking with a dose of 560 mg/kg. In subsequent experiments, the backward walking incidence was examined only at 20, 30 and 40 min after dosing with L-5HTP, and all comparative evaluations were made with the obtained peak values.

**Backward walking after dosing with D-5HTP or L-DOPA:** No backward walking
was noted in mice after dosing with D-5HTP at doses of 100 and 320 mg/kg or L-DOPA at doses of 100, 320 and 560 mg/kg.

Inhibition of L-5HTP-induced backward walking by DL-α-methyl-DOPA: L-5HTP-induced backward walking in mice was inhibited by 1 hr pretreatment of the animals with DL-α-methyl-DOPA, an inhibitor of aromatic amino acid decarboxylase. The inhibitory effect was in a dose dependent manner, and almost complete inhibition was noted at a dose of 320 mg/kg of DL-α-methyl-DOPA (Fig. 2).

Potentiation of L-5HTP-induced backward walking by a monoamine oxidase inhibitor: Neither small doses of L-5HTP (3.2–32 mg/kg), nor a monoamine oxidase inhibitor (MAOI), tranylcypromine 10 mg/kg or pargyline 100 mg/kg, produced the backward walking by itself. However, when these treatments were combined, the backward walking was evoked, as shown in Fig. 3.

Backward walking after the intracerebral injection of serotonin: Backward walking was examined at various times after intracerebral injection of 5-HT to mice. Results are shown in Fig. 4. Injection of 5-HT in doses of 10 and 100 μg/animal caused the backward walking in one and five of 10 animals, respectively. After intracerebral

![Graph 1: Relation of the doses of L-5HTP to incidences of backward walking in mice.](image1)

![Graph 2: Inhibition of L-5HTP-induced backward walking by DL-α-methyl-DOPA in mice.](image2)

![Graph 3: Potentiation of L-5HTP-induced backward walking by a MAOI.](image3)
injection of saline instead of 5-HT, the backward walking was not observed in mice. When mice were given 5-HT 10 μg/animal 1 hr after the i.p. injection of tranylcypromine 10 mg/kg, the backward walking was markedly potentiated and persisted over 2 hr.

Potentiating effects of 5-HT uptake inhibitors on the backward walking: Zimelidine (8) and clomipramine (9) were used as inhibitors of neuronal uptake of 5-HT. Desipramine, which has been described to be an inhibitor selective for noradrenaline (NA) uptake (9, 10), was used for comparison. Results are shown in Table 1.

L-5HTP injection at a dose of 80 mg/kg to

![Figure 4](image)

**Fig. 4.** Backward walking after intracerebral administration of 5-HT. The results were expressed by the percentages of animals showing the backward walking. Tranylcypromine 10 mg/kg was given i.p. 1 hr before the intracerebral injection of 5-HT. (A), (△): 5-HT 10 μg/animal alone, (▲): tranylcypromine 10 mg/kg+5-HT 10 μg/animal, (B), (○): 5-HT 100 μg/animal alone, (●): tranylcypromine 10 mg/kg+5-HT 100 μg/animal. Ten mice were included in each group.

### Table 1. Potentiating effects of drugs on backward walking

| Dose (mg/kg) | Drug | Zimelidine | Clomipramine | Desipramine |
|-------------|------|------------|---------------|-------------|
|             |      | p.o.       | i.p.          | p.o.        | i.p.        |
| 0           | 0    | 0          | 0             | 0           | 0           |
| 1           | 0    | 10         | 10            | 10          | –           |
| 3.2         | 20   | 20         | 20            | 10          | 0           |
| 10          | 30   | 30         | 10            | 10          | 10          |
| 32          | 90   | 90         | 0             | 10          | 40          |
| 100         | –    | –          | 80            | –           | 0*          |

Drugs were given p.o. 60 min or i.p. 30 min before dosing with L-5HTP in a dose of 80 mg/kg. Control group was given the vehicle only. Values are peak effects in percentages of animals showing the backward walking at 20, 30 or 40 min after dosing with L-5HTP. Each group comprised 10 mice. –: not tested, *: six of 10 mice died.
mice produced only few incidences of backward walking. The effect of L-5HTP in this threshold dose was markedly potentiated by pretreatment of the animals with zimelidine or clomipramine. Zimelidine was more effective than clomipramine when given by p.o. route, while both drugs were almost equipotent in efficacy by i.p. route. Neither drug caused backward walking by itself even in large doses. Desipramine did not show more than 40% potentiation in backward walking incidence in i.p. doses up to 100 mg/kg, and was almost ineffective in p.o. route.

Inhibiting effects of drugs on the backward walking

| Drug (i.p.) | Dose (mg/kg) | N | Backward walking (%) |
|------------|--------------|---|---------------------|
| Control    | -            | 10| 90                  |
| Cyproheptadine | 1            | 10| 90                  |
|            | 3.2          | 10| 40*                 |
|            | 10           | 10| 20*                 |
|            | 32           | 10| 0*                  |
| Control    | -            | 10| 80                  |
| Methysergide | 1            | 15| 67                  |
|            | 3.2          | 15| 60                  |
|            | 10           | 15| 27*                 |
|            | 32           | 15| 7*                  |
| Control    | -            | 10| 80                  |
| Fluphenazine | 0.1          | 10| 60                  |
|            | 0.32         | 10| 0*                  |
|            | 1            | 10| 0*                  |
| Control    | -            | 10| 60                  |
| Haloperidol | 0.32         | 10| 0*                  |
|            | 1            | 10| 0*                  |
| Control    | -            | 10| 100                 |
| Chlorpromazine | 1           | 10| 70                  |
|            | 3.2          | 10| 50*                 |
|            | 10           | 10| 0*                  |
| Control    | -            | 10| 80                  |
| Diphenhydramine | 10          | 10| 50                  |
|            | 32           | 10| 80                  |
| Control    | -            | 10| 80                  |
| Phentolamine | 1            | 10| 70                  |
|            | 10           | 10| 40                  |
| Control    | -            | 10| 80                  |
| DL-propranolol | 1           | 10| 50                  |
|            | 10           | 10| 50                  |
| Control    | -            | 10| 70                  |
| Atropine   | 1            | 10| 60                  |
|            | 3.2          | 10| 50                  |

Drugs were given i.p. 30 min before dosing with L-5HTP in a dose of 500 mg/kg. Control group was given a vehicle instead of a drug. Values are peak percentages of animals showing the backward walking at 20, 30 and 40 min after dosing with L-5HTP. N=number of animals. *P<0.05: significantly different from the corresponding control group.
walking: Table 2 shows the effect of various kinds of drugs on the backward walking induced by L-5HTP at a dose of 500 mg/kg which produced the backward walking at a high rate in control animals. Cyproheptadine and methysergide, 5-HT receptor blockers, significantly inhibited the backward walking in doses as small as 3.2 to 10 mg/kg. Dopamine (DA) receptor blockers, fluphenazine, haloperidol and chlorpromazine markedly to completely inhibited the backward walking, in small doses. However, diphenhydramine, phentolamine, DL-propranolol and atropine had no significant effects on the backward walking, even in large doses.

DISCUSSION

The dosing of 5-HTP produces an increase of the brain 5-HT (1, 4). The present results indicate that the brain 5-HT is primarily responsible for induction of the backward walking: 1) Neither the optical isomer D-5HTP which cannot be converted to 5-HT in animals, nor L-DOPA, a precursor of catecholamines, induced such behaviour, 2) inhibition by DL-α-methyl-DOPA of the aromatic amino acids decarboxylase, which converts L-5HTP to 5-HT, suppressed the backward walking, whereas a MAOI, tranylcypromine or pargyline, potentiated the backward walking, 3) the backward walking was inhibited by the 5-HT receptor blockers, cyproheptadine and methysergide, and 4) the intracerebral injection of 5-HT in mice induced the backward walking which was potentiated by a MAOI. However, at present, we have no explanation of why the larger dose of 5-HT caused a lower incidence of backward walking.

In addition, the brain DA also appears to be involved in the backward walking induced by L-5HTP, because the behaviour was inhibited by the DA receptor blockers, while other blockers including α- and β-adrenergic receptor blockers, an antihistamine, and an anticholinergic agent had no effect. The results are compatible with the findings of others that the central catecholamines are involved in the head-twitch induced by L-5HTP in mice (11). Grahame-Smith (12, 13) and Green and Grahame-Smith (14) have also reported that the central DA are required for the hyperactivity induced by L-tryptophan and a MAOI. On the other hand, Wray (15), and Phillips and Wray (16) reported that the brain DA, but not 5-HT, plays a role in the levallorphan-induced stereotypy in rats, including the backward walking.

To study the potentiating effect of 5-HT uptake inhibitors, L-5HTP was used at the dose of 80 mg/kg, this being the threshold dose required to produce backward walking. Zimelidine and clomipramine markedly potentiated the backward walking in a dose-related manner, whereas the effect of desipramine was weak and did not exceed 40% even at the dose of 32 mg/kg i.p. which appeared to be toxic. Both zimelidine (8, 17) and clomipramine (9) have been reported to be selective inhibitors of the neuronal uptake of 5-HT. On the other hand, desipramine have been reported to be a selective inhibitor of neuronal uptake of NA (9, 10). Therefore, the potentiating effect of zimelidine and clomipramine on the backward walking may be due to the increased concentration of 5-HT at synaptic sites. In the present results, zimelidine and clomipramine were almost equipotent when given i.p., but zimelidine was more potent than clomipramine when given p.o. Thus, zimelidine appears to be more easily absorbed from the digestive tract.

The injection of large doses of L-5HTP can induce head-twitch (1, 4). The head-twitch has been reported to be produced by the central action of 5-HT, and is used as a behavioural model to evaluate the central
serotonergic activity of drugs (1, 4, 5, 6, 8). In this model the results can be quantified by counting the number of head-twitches. However, careful watch is requested in this model, because the head-twitch is a momentary movement. By contrast, it is easy to note the occurrence of backward walking. When the animals once began to walk backwards, they walked at least over 10 cm. Some animals even continuously walked a longer distance, therefore one had to reverse the direction of animals to keep them on the table. Those animals with unilateral abduction of hind-legs walked backward in a circle. Although the backward walking induced by L-5HTP has been described by other workers (18), the pharmacological nature of this model has not been studied. From the results described in the present paper, the backward walking seems to depend primarily on the central serotonergic activity. This behaviour, therefore, could be an useful parameter in assessing the activity of drugs inhibiting the neuronal 5-HT receptors in the central nervous system. However, it must be kept in mind that some dopaminergic drugs can also modify this behaviour.

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