Pharmacological Mechanism in Slip-Down Behavior of Mice

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Slip-down from a raised platform was previously found in mice treated with
morphine, and this behavior was also recognized in mice treated with a
monoamine releaser methamphetamine. Pharmacological examination on the
slip-down indicated that the behavior was induced by receptor stimulations by D₂
agonist PPHT and 5HT-2 agonist DOI. In mice treated with PPHT, antagonists
of D₁, α_2 , 5HT-2 and opioid mu and kappa suppressed the behavior. In mice
treated with DOI, antagonists of D₁, α_2 , 5HT-1A, 5HT-3 and opioid mu and delta
suppressed the behavior. These present findings suggest that the slip-down was
mainly induced by opioid mu receptor activity regulated with monoamine activ-
ities. When the slip-down is considered as an anxious behavior, it may be also
suggested that the anxiety induced by 5-HT activities furthermore stimulated the
behavior via the other opioid receptor activities. ——— slip-down behavior;
mice; anxiety; opioid receptor; neurotransmitter activities

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It was previously reported that morphine induces an abnormal behavior, slip-down from a raised platform, on mice (Hui and Roberts 1975). We occasionally recognized that mice treated with a monoamine releaser methamphetamine (MAP) also showed the behavior. We have been very interested in relations of opioid receptor activities and the other neurotransmitter receptor activities in the behavior. In this paper, we investigate the pharmacological mechanism of the behavior.

MATERIALS AND METHODS

Animals

Male ddY mice, 8 weeks-old (Japan SLC, Inc., Shizuoka) were prepared. Each 5 of them were housed in a plastic cage (338×140×225 mm) with free access to food (CE-7: Clea Japan Co., Tokyo) and water in a tapping plastic bottle. The animal room was kept at 21-25°C with 50-60% humidity and illuminated from 7:00 to 19:00. All of the experiments were

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carried out under the control of the Ethics Committee for Animal Experiments of Akita University School of Medicine.

Method detecting the slip-down and the effect of MAP

MAP was purchased from Dainippon Seiyaku Co. (Osaka) and was prepared with water. A raised platform having 10 cm diameter and 20 cm height was prepared. Five mice in a cage were intraperitoneally injected with 1000, 100 or 10 $\mu\text{g}/\text{kg}$ of MAP, or 100 μl of physiological saline (PS) as a control. Twenty minutes after the injection, the mice were individually placed on the platform and the slip-down from the platform was investigated for 5 minutes.

Effects of antagonists on the slip-down of mice treated with MAP

Antagonists, SCH-23390 (D_1), sulpiride (D_2), prazosin (α_1), yohimbine (α_2), propranolol (β), NAN190 (5-HT1A), ketanserin (5-HT2) and LY-278, 584 (5-HT3) were purchased from Sigma Co. (St. Louis, MO, USA) and were prepared with water. A group of 5 mice were intraperitoneally injected with 100, 10 or 1 $\mu\text{g}/\text{kg}$ of one of the antagonists, or 100 μl of PS as a control. Twenty minutes after the injection, these groups of mice were intraperitoneally treated with 1 $\mu\text{g}/\text{kg}$ of MAP, and then, the slip-down of these mice was individually investigated 20 minutes after the MAP treatment.

Agonists inducing the slip-down

Agonists, SKF-38393 (D_1), PPHT hydrochloride (D_2), methoxamine (α_1), clonidine (α_2), isoproterenol (β), 8-Hydroxy-DPAT (5-HT1A), DOI hydrochloride (5-HT2) and 2-methylserotonin (5-HT3) were also purchased from Sigma Co. and were prepared with water. One hundred, 50 or 25 $\mu\text{g}/\text{kg}$ of one of the agonists, or 100 μl of PS as a control was injected to a group of five mice, and then, the slip-down of these mice was individually investigated.

Effects of antagonists on the slip-down of mice treated with PPHT

One hundred, 10 or 1 $\mu\text{g}/\text{kg}$ of the antagonist of D_1 , α_1 , α_2 , β , 5-HT1A, 5-HT2 or 5-HT3, 1000, 100 or 10 $\mu\text{g}/\text{kg}$ of antagonist of opioid mu, delta or kappa (naloxone, ICI 174864, naltorphine; Sigma Co.), or 100 μl of PS as a control was injected to a group of five mice. Twenty minutes after the injection, 100 $\mu\text{g}/\text{kg}$ of PPHT was treated to these mice, and then, the slip-down of the mice was individually investigated.

Effects of antagonists on mice treated with DOI

One hundred, 10 or 1 $\mu\text{g}/\text{kg}$ of the antagonist of D_1 , D_2 , α_1 , α_2 , β , 5-HT1A or 5-HT3, 1000, 100 or 10 $\mu\text{g}/\text{kg}$ of the antagonist of opioid mu, delta or kappa, or 100 μl of PS as a control was injected to a group of five mice. Twenty minutes after the injection, 100 $\mu\text{g}/\text{kg}$ of DOI was treated to these mice, and then, the slip-down of the mice was individually investigated.

Statistical analysis

The analysis was performed with Fisher's exact probability test.

RESULTS

MAP induced the slip-down at the dose of 1 mg/kg (Table 1). Stereotyped behaviors: continuous sniffing and circling were not found in the mice treated with MAP at any doses. The slip-down induced by MAP was suppressed by the D_1 , D_2 , α_2 , 5-HT1A, 5-HT2 or 5-HT3 receptor antagonists (Table 2). D_2 agonist PPHT 100 $\mu\text{g}/\text{kg}$ and 5-HT2 agonist DOI 100 $\mu\text{g}/\text{kg}$ induced the slip-down on mice, but the other agonists did not (Table 3). The slip-down induced by PPHT was suppressed by the D_1 , α_2 , 5-HT2, opioid mu or kappa antagonist (Table 4). The slip-down induced by DOI was suppressed by the D_1 , α_2 , 5-HT1A, 5-HT3, opioid mu or delta antagonist (Table 5).

TABLE 1. *Effect of methamphetamine on the slip-down behavior*

Mice showing the ship-down Dose ($\mu\text{g/kg}$) of methamphetamine			
1000	100	10	(Control) PS
5*	0	0	0

A group of five mice were intraperitoneally injected with methamphetamine solution at these doses. Twenty minutes after the treatments, mice were individually placed on a platform and were investigated the slip-down.

PS; physiological saline.

* $p=0.0396<0.05$ compared to the control (Fisher's exact probability test).

TABLE 2. *Effects of antagonists on the slip-down behavior induced by methamphetamine*

Antagonist	Mice showing the ship-down Dose ($\mu\text{g/kg}$)		
	100	10	1
D ₁ : SCH-23390	0*	0*	5
D ₂ : Sulpiride	0*	0*	5
α_1 : Prazosin	5	5	5
α_2 : Yohimbine	0*	0*	5
β : Propranolol	5	5	5
5-HT _{1A} : NAN 190	0*	5	5
5-HT ₂ : Ketanserin	0*	5	5
5-HT ₃ : LY-278, 584	0*	5	5
(Control) PS		5	

A group of five mice were intraperitoneally injected with each antagonist, and also treated with 1 mg/kg of methamphetamine 20 minutes after the injection. PS, physiological saline.

* $p=0.0396<0.05$ compared to the control (Fisher's exact probability test).

DISCUSSION

Behaviorally, the slip-down is different from the increasing of ambulation of mice, because the ambulation induced by MAP increases on the place with the diameter of 15 cm and above (Hirabayashi et al. 1991). Fall-down in cliff avoidance reaction test looks like the slip-down, but the behavior was not found in mice treated with MAP (Yoshida et al. 2000). As behaviors induced by MAP are affected by

TABLE 3. *Agonists inducing the slip-down*

Agonist	Mice showing the ship-down Dose ($\mu\text{g/kg}$)		
	100	50	25
D ₁ : SKF-38393	0	0	0
D ₂ : PPHT	5*	0	0
α_1 : Methoxamine	0	0	0
α_2 : Clonidine	0	0	0
β : Isoproterenol	0	0	0
5-HT _{1A} : DPAT	0	0	0
5-HT ₂ : DOI	5*	0	0
5-HT ₃ : 2-methylserotonin	0	0	0
(Control) PS		0	

Each of agonists was treated to a group of five mice.

PS; physiological saline.

* $p=0.0396<0.05$ compared to the control (Fisher's exact probability test).

TABLE 4. *Effects of antagonists on the slip-down behavior induced by D₂ agonist PPHT*

Antagonist	Mice showing the ship-down Dose ($\mu\text{g/kg}$)		
	100	10	1
D ₁	0*	5	5
α_1	5	5	5
α_2	0*	0*	5
β	5	5	5
5-HT _{1A}	5	5	5
5-HT ₂	0*	0*	5
5-HT ₃	5	5	5

Opioid

Antagonist	1000	100	10
μ : naloxone	0*	5	5
δ : ICI 174864	5	5	5
κ : nor-binaltorphimine	0*	5	5
(Control) PS		5	

A group of five mice were injected with each antagonist, and also treated with 100 $\mu\text{g/kg}$ of PPHT 20 minutes after the injection.

* $p=0.0396<0.05$ compared to the control (Fisher's exact probability test).

situations in which the mice are (Masuda et al. 1996), the impairment of the fall-down in cliff avoidance reaction may be related with the

TABLE 5. *Effects of antagonists on the slip-down behavior induced by 5-HT2 agonist DOI*

Antagonist	Mice showing the slip-down Dose ($\mu\text{g/kg}$)		
	100	10	1
D ₁	0*	5	5
D ₂	5	5	5
α_1	5	5	5
α_2	0*	0*	5
β	5	5	5
5-HT1A	0*	5	5
5-HT3	0*	5	5
<i>Opioid</i>			
Antagonist	1000	100	10
mu	0*	5	5
delta	0*	0*	5
kappa	5	5	5
(Control) PS		5	

A group of five mice were injected with each antagonist, and also treated with 100 $\mu\text{g/kg}$ of DOI 20 minutes after the injection.

* $p=0.0396<0.05$ compared to the control (Fisher's exact probability test).

width of the place in which the mice were. Finally, the slip-down of mice treated with MAP may reflect the anxiety in narrow space.

Pharmacologically, the present findings indicate that the slip-down was induced via two pathways, the D₂ and 5-HT₂ receptor stimulations. The D₂ pathway stimulated opioid mu and kappa receptor activities via D₁, α_2 and 5-HT₂ receptor activities, and the 5-HT₂ pathway stimulated opioid mu and delta activities via D₁, α_2 , 5-HT_{1A} and 5-HT₃ receptor activities. It looks that D₁ and α_2 receptor activities regulated opioid mu receptor activities and 5-HT receptor activities regulated kappa and delta receptor activities. In fact, dopamine stimulates opioid mu receptor activity (Cabocho et al. 1991), and α_2 -adrenoceptor activity stimulates opioid mu and kappa receptor activities (Schreiber et al. 2002). As the slip-down induced by morphine is affected by naloxone treatment (Hui and Roberts 1975), the slip-

down induced by MAP may be mainly driven with the opioid mu receptor activity. Otherwise, 5-HT activities temporally induce anxiety under dopamine receptor activities (Belzung et al. 2001). The anxiety may furthermore stimulate the slip-down via the other opioid receptor activities. Relations of 5-HT activities and opioid receptor activities, however, have not been clear.

Not only morphine but also MAP sometimes induces various psychotic symptoms on humans. Lately, it was reported that D₂ and 5-HT₂ receptor activities have a relation to MAP psychosis (Iyo et al. 1993). These and the present findings suggest that some mechanisms of MAP psychosis are common to those of morphine psychosis. Although it is unclear which symptom of the patients corresponds to the slip-down of mice, the slip-down may be an interesting method examining the common mechanisms of the psychoses.

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