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σ_1 Receptor subtype is involved in the relief of behavioral despair in the mouse forced swimming test

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Abstract

The immobility time in the mouse forced swimming test was dose-dependently reduced by σ_1 receptor agonists, such as 1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl)piperazine dihydrochloride (SA4503) and (+)-pentazocine, and non-specific σ receptor agonists, such as 1,3-di(2-tolyl)guanidine (DTG) and (+)-*N*-cyclopropyl-methyl-*N*-methyl-1,4-diphenyl-1-yl-but-3-en-1-ylamine hydrochloride (JO-1784). On the other hand, pre-treatment with *N*,*N*-dipropyl-2-[4-methoxy-3-(2-phenylethoxy)phenyl]ethylamine monohydrochloride (NE-100), a putative σ_1 receptor antagonist, completely antagonized the SA4503-, (+)-pentazocine- and DTG-induced reductions in immobility time. Such phenomena indicate that σ receptor agonists alleviate behavioral despair. In addition, these antidepressive effects involve mainly the σ_1 receptor subtype.

Keywords: σ_1 Receptor agonist; Antidepressive effect; Forced swimming test; SA4503; (Mouse)

1. Introduction

The σ receptors have been reported to modulate neurotransmission in the central nervous system. Interestingly, the σ receptors were reported to be involved in noradrenergic and/or glutamatergic transmission (Kinouchi et al., 1989; Rogers and Lemaire, 1991; Fletcher et al., 1993; Ellis and Davies, 1994), in addition to dopaminergic and/or cholinergic transmission (Matsuno et al., 1993, 1994, 1995a,b). For example, σ receptor ligands have been reported to inhibit [^{3}H]norepinephrine uptake in rat brain synaptosomes (Kinouchi et al., 1989; Rogers and Lemaire, 1991). In addition, (+)-pentazocine, a prototype σ_1 receptor ligand (Itzhak, 1994), has been reported to augment [^{3}H]norepinephrine release from rat cortical slices (Kinouchi et al., 1989). In contrast, σ receptor ligands were reported to inhibit glutamate release from rat striatal slices (Ellis and Davies, 1994). Moreover, 1,3-di(2-tolyl)guanidine (DTG), a non-specific σ receptor ligand (Itzhak, 1994), blocked *N*-methyl-D-aspartate (NMDA) receptor-mediated responses in both rat and mouse cultured neurons (Fletcher et al., 1993).

The central noradrenergic and/or glutamatergic systems play important roles in the pathophysiology of depression. The hypothesis that the activation of norepinephrine transmission is involved in the relief of behavioral despair has been well documented (Bunney and Davies, 1965). In addition, it has been shown that a competitive and a non-competitive NMDA receptor antagonists reduced the immobility time and mimicked the effects of clinically effective antidepressant drugs in the behavioral depression models (Trullas and Skolnick, 1990). Thus, these findings raised the possibility that the σ receptors might be involved in the pathophysiology of affective disorders, or might be a potential target for antidepressant treatment.

Recent binding studies have shown that σ receptors, particularly the σ_1 receptor subtype, may be associated with G-proteins, particularly the $G_{i/o}$ types (Itzhak and Khouri, 1988; Beart et al., 1989; Itzhak and Stein, 1991; Connick et al., 1992). Based on these findings, σ_1 receptor agonist has been reported to show a decrease of binding affinity in the presence of guanine nucleotides, whereas the binding affinity of σ_1 receptor antagonist was unaffected (Beart et al., 1989; Connick et al., 1992; Tanaka

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et al., 1995; Matsuno et al., 1996a). Similar to this in vitro classification, pharmacological studies also have shown that 1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl)-piperazine dihydrochloride (SA4503) and (+)-pentazocine might be σ_1 receptor agonists, DTG and (+)-*N*-cyclopropylmethyl-*N*-methyl-1,4-diphenyl-1-yl-but-3-en-1-ylamine hydrochloride (JO-1784) might be non-specific σ receptor agonists, whereas haloperidol and *N*,*N*-dipropyl-2-[4-methoxy-3-(2-phenylethoxy)phenyl]-ethylamine monohydrochloride (NE-100) might be σ_1 receptor antagonists (Junien et al., 1991; Monnet et al., 1992; Okuyama et al., 1993; Matsuno et al., 1993, 1996b).

Thus, to examine whether σ receptor agonists alleviate behavioral despair, this study was designed to investigate the effects of SA4503 and (+)-pentazocine, σ_1 receptor agonists, and DTG and JO-1784, non-specific σ receptor agonists, on the immobility time in the mouse forced swimming test. We also examined whether these effects were antagonized by the pre-administration of NE-100, a putative σ_1 receptor antagonist.

2. Materials and methods

2.1. Animals

Five male ddY mice (Nihon SLC, Shizuoka, Japan), weighing 20 ~ 34 g, were housed in a plastic cage, with free access to food and water, in a controlled environment (23 ± 1°C and 55 ± 10% humidity), with a 12-h light-dark cycle (light on between 07:00 and 19:00 h). The mice were used following at least 7 days' adaptation to laboratory conditions.

2.2. Drugs

SA4503 (Matsuno et al., 1996a,b), (+)-pentazocine, JO-1784 (Roman et al., 1990) and NE-100 (Okuyama et al., 1993) were synthesized by Santen Pharmaceutical. DTG and mianserin were purchased from Research Biochemicals. SA4503, NE-100 and mianserin were suspended in 1% methylcellulose solution and administered orally. JO-1784 was dissolved in saline, and administered subcutaneously and intraperitoneally, respectively. (+)-Pentazocine and DTG were initially dissolved in 1 N HCl and neutralized with 1 N NaOH, and then diluted in saline, and administered subcutaneouly. All drugs were given at a dose of 0.1 ml/10 g body weight. The drug dose calculations were based on the salt form.

2.3. Experimental protocol

2.3.1. Forced swimming test

The total duration of immobility was measured using a modified version of the behavioral despair test (Porsolt et al., 1977a,b). Each mouse was placed individually in a glass cylinder (height 21 cm, diameter 12 cm) containing 9 cm of water at 22–23°C for 15 min (pre-test). 24 h after the pre-test, each mouse was again placed in the glass cylinders, and the immobility time was recorded for the last 5 min of a 6-min forced swimming test, because the vigorous activity was observed during the first 1 min. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water making only those movements necessary to keep its head above water. To assess the effects of single administration of SA4503, (+)-pentazocine, DTG, JO-1784 or NE-100, these agents were administered 30 min before the experiment on day 2. To evaluate antagonism with NE-100, this agent was administered 15 min before the administration of SA4503, (+)-pentazocine or DTG.

2.3.2. Spontaneous locomotor activity

Locomotor activity was measured in a transparent acrylic cage (22 × 38 × 20 cm), surrounded by a photocell-equipped instrument (SCANET model MV-10, MATYS, Japan). The photocell was set at a 6-mm interval. Mice were gently placed into the test cage 30 min after drug administration, and their locomotor activities were counted for 5 min from 1 min after placed the mice into the test cage.

2.4. Data analysis

Results are expressed as means ± S.E.M. Comparisons between the treatment groups and control were performed by analysis of variance (ANOVA) followed by Dunnett's multiple range comparison test and the differences between the two groups were analyzed using Student's unpaired *t*-test.

3. Results

3.1. Effects of σ receptor agonists and antagonist on the immobility time in the mouse forced swimming test

A novel σ_1 receptor agonist, SA4503, significantly shortened the immobility time in the mouse forced swimming test at doses of 0.1 and 1.0 mg/kg ($F(5,73) = 5.66$, $P < 0.01$) (Table 1). Similarly, a prototype σ_1 receptor agonist, (+)-pentazocine, dose-dependently reduced the immobility time in the mouse forced swimming test ($F(3,42) = 4.14$, $P < 0.05$) (Table 1). On the other hand, DTG and JO-1784, non-selective σ receptor agonists, also reduced the immobility time (DTG: $F(2,30) = 7.25$, $P < 0.01$, JO-1784: $F(3,54) = 5.07$, $P < 0.01$) (Table 1). Significant reductions in immobility time by each σ receptor agonist were observed at the highest doses used in the present study (Table 1). NE-100, a putative σ_1 receptor antagonist, alone did not affect the immobility time ($F(2,30) = 0.04$, $P > 0.05$) (Table 1). Mianserin signifi-

Table 1
Effects of σ receptor agonists and antagonist on the immobility time during the last 5 min of a 6-min forced swimming test in mice

Treatment	Dose (mg/kg)	n	Immobility time (s)
σ_1 Receptor agonist			
Vehicle (p.o.)		22	214.8 ± 10.0
SA4503 (p.o.)	0.05	11	188.2 ± 18.6
	0.1	12	152.0 ± 23.0 [a]
	0.25	12	183.3 ± 15.9
	0.5	11	172.4 ± 12.3
	1.0	11	111.6 ± 14.5 [b]
Vehicle (s.c.)		12	226.7 ± 12.2
(+)-Pentazocine (s.c.)	1.0	11	215.2 ± 12.1
	2.5	11	171.3 ± 19.5
	5.0	12	160.3 ± 19.0 [a]
Non-specific σ receptor agonist			
Vehicle (s.c.)		11	237.8 ± 6.8
DTG (s.c.)	2.5	11	215.2 ± 15.4
	5.0	11	155.4 ± 21.6 [b]
Vehicle (i.p.)		20	207.2 ± 10.3
JO-1784 (i.p.)	2.5	13	190.8 ± 17.6
	5.0	12	162.9 ± 19.3
	10.0	13	131.3 ± 16.1 [b]
σ_1 Receptor antagonist			
Vehicle (p.o.)		11	228.7 ± 14.7
NE-100 (p.o.)	0.25	11	229.5 ± 10.7
	0.5	11	224.7 ± 11.2
Atypical antidepressant			
Vehicle (p.o.)		12	232.4 ± 10.7
Mianserin (p.o.)	20.0	13	167.1 ± 17.6 [b]

Mice were subjected to 15 min forced swimming 24 h before the test. Each ligand was administered 30 min before the swimming test on day 2. The results are expressed as mean ± S.E.M.
[a] $P < 0.05$ and [b] $P < 0.01$ as compared with each vehicle.

cantly reduced the immobility time at a dose of 20.0 mg/kg.

3.2. Antagonism by NE-100 of the σ receptor agonists-induced reductions in the immobility time in the mouse forced swimming test

Pre-administration of NE-100 completely antagonized the SA4503-, (+)-pentazocine- and DTG-induced reductions in immobility responses (SA4503: $F(1,18) = 27.50$, $P < 0.01$; (+)-pentazocine: $F(1,26) = 13.92$, $P < 0.01$; DTG: $F(1,24) = 12.42$, $P < 0.01$) (Table 2). Similar to Table 1, NE-100 alone did not affect the immobility time (Table 2).

3.3. Effects of σ receptor agonists and antagonist on the spontaneous locomotor activity in the mice

As the changes in the locomotor activity could influence the measurement of immobility time, we examined the effects of the σ receptor agonists and antagonist on the spontaneous locomotor activity in the mice. As shown in Table 3, none of the compounds administered affected the spontaneous locomotor activity in mice during 5 min (31–36 min after administration).

Table 2
Antagonism by NE-100 of the SA4503-, (+)-pentazocine- and DTG-induced reductions in immobility time during the last 5 min of a 6-min forced swimming test in mice

Treatment (dose (mg/kg))	n	Immobility time (s)
Vehicle + vehicle	10	207.8 ± 18.4
Vehicle + SA4503 1.0	10	118.7 ± 17.6 [a]
NE-100 0.5 + SA4503 1.0	10	230.3 ± 12.0 [b]
NE-100 0.5 + vehicle	8	213.4 ± 14.4
Vehicle + vehicle	14	208.4 ± 10.7
Vehicle + (+)-pentazocine 5.0	14	139.2 ± 12.8 [a]
NE-100 0.5 + (+)-pentazocine 5.0	14	200.5 ± 10.3 [b]
NE-100 0.5 + vehicle	8	230.8 ± 14.9
Vehicle + vehicle	13	229.4 ± 11.6
Vehicle + DTG 5.0	13	158.1 ± 12.7 [a]
NE-100 0.5 + DTG 5.0	13	215.1 ± 10.0 [b]
NE-100 0.5 + vehicle	8	227.9 ± 13.2

Mice were subjected to 15 min forced swimming 24 h before the test. SA4503 was orally administered 30 min before the swimming test on day 2. (+)-Pentazocine and DTG were subcutaneously injected 30 min before the swimming test on day 2. NE-100 was administered orally 15 min before administration of SA4503, (+)-pentazocine or DTG. The results are expressed as mean ± S.E.M.
[a] $P < 0.01$ as compared with each vehicle + vehicle.
[b] $P < 0.01$ as compared with the vehicle + SA4503, vehicle + (+)-pentazocine or vehicle + DTG

Table 3
Effects of σ receptor agonists and antagonist on the locomotor activity in mice

Treatment	Dose (mg/kg)	n	Locomotor activity counts
σ_1 Receptor agonist			
Vehicle (p.o.)		7	2126 ± 391
SA4503	0.1	7	1943 ± 373
	1.0	7	2286 ± 312
Vehicle (s.c.)		7	1979 ± 430
(+)-Pentazocine	5.0	7	1572 ± 61
Non-specific σ receptor agonist			
Vehicle (s.c.)		7	1979 ± 430
DTG	5.0	7	2188 ± 255
Vehicle (i.p.)		7	2118 ± 100
JO-1784	10.0	7	2290 ± 135
σ_1 Receptor antagonist			
Vehicle (p.o.)		7	2126 ± 391
NE-100	0.5	7	1900 ± 264

Mice were gently placed into the test cage 30 min after drug administration, and their locomotor activities were counted for 5 min from 1 min after placed the mice into the test cage. The results are expressed as mean ± S.E.M.

4. Discussion

Binding studies have demonstrated that norepinephrine or serotonin uptake inhibitors potently inhibited [^{3}H](+)-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine ([^{3}H](+)-3-PPP) binding to σ receptors in the rat brain (Schmidt et al., 1989). In addition, monoamine oxidase inhibitors have also been reported to displace the [^{3}H](+)-3-PPP binding to σ receptors in C57BL/6 mouse brain (Itzhak and Kassim, 1990). Thus, it is possible that σ receptors play an important role in the pathophysiology of depression. Interestingly, we found that σ receptor agonists significantly relieved the immobility response in the present behavioral despair model.

SA4503 and (+)-pentazocine showed high affinity for the σ_1 receptor subtype, but not the σ_2 receptor subtype (Itzhak, 1994; Chaki et al., 1994; Matsuno et al., 1996a). In addition, the reduction in duration of immobility elicited by DTG, a non-specific σ receptor agonist (Itzhak, 1994), was completely antagonized by NE-100, a putative σ_1 receptor antagonist (Okuyama et al., 1993; Chaki et al., 1994). Thus, we suggest that the σ_1 receptor subtype plays an important role in the behavioral response to depression and that drugs which have agonistic properties for the σ_1 receptor subtype showed antidepressive effects. A few physiological functions of the σ receptor subtypes have been clarified so far. An antagonist of the σ_1 receptor subtype was reported to have antipsychotic effects in mice and rats (Okuyama et al., 1993). On the other hand, our previous study showed that an agonist of the σ_1 receptor subtype improved the drug-induced memory impairment in rats (Matsuno et al., 1993, 1994, 1995b,1996b). In the present study, we further demonstrated that the antidepressive effect is a novel physiological function of the σ_1 receptor subtype. Thus, σ_1 receptor agonists may be useful for treating patients with not only senile dementia, but also depression. In contrast, another possibility that the σ_2 receptor subtype also contributes to the antidepressive effects of σ receptor agonists was considered, because DTG was reported to bind to not only the σ_1 receptor subtype, but also the σ_2 receptor subtype (Quirion et al., 1992; Itzhak, 1994). The involvement of the σ_2 receptor subtype in the antidepressive effects of σ receptor agonists remains unclear, because there are no specific ligands for the σ_2 receptor subtype so far.

In conclusion, the present results suggest that the σ receptor agonists have antidepressive properties in rodents, and that the σ_1 receptor subtype may be mainly involved in the behavioral response to depression.

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