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Possible Involvement of a σ Receptor Subtype in the Neck Dystonia in Rats

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NAKAZAWA, M., T. KOBAYASHI, K. MATSUNO AND S. MITA. *Possible involvement of a σ receptor subtype in the neck dystonia in rats*. PHARMACOL BIOCHEM BEHAV **62**(1)123–126, 1999.—To clarify which subtype of σ receptors is involved in the σ receptor-mediated neck dystonia in rats, we examined whether 1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl)piperazine dihydrochloride (SA4503), a selective σ_1 receptor agonist, and 1,3-di-(2-tolyl)guanidine (DTG), a σ_1 and σ_2 receptor agonist, induce neck dystonia in rats. Microinjection of SA4503 into the red nucleus of rat brain scarcely produced neck dystonia at the concentration of 10 nmol/0.5 μl. On the contrary, DTG produced significant dystonia at a concentrations of more than 5 nmol/0.5 μl. These results indicate that the σ_2 receptor subtype, but not σ_1 receptor subtype, may play an important role in the σ receptor-mediated neck dystonia in rats. © 1998 Elsevier Science Inc.

σ_1 Receptor subtype σ_2 Receptor subtype Neck dystonia Red nucleus SA4503 DTG

EVIDENCE that σ receptors may be involved in the regulation of movement and posture stems from both behavioral and biochemical studies. For example, σ receptors are concentrated in brain structures that control movement, such as the red nucleus and substantia nigra (3,7). In addition, the unilateral microinjection of σ receptor ligands, such as 1,3-di(2-tolyl)guanidine (DTG), haloperidol, (+)-*N*-allylnormetazocine [(+)-SKF-10,047] and (+)-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine [(+)-3-PPP], into the red nucleus induces neck dystonia in rats (1,10,19,27,28). The behavioral potency of these σ receptor ligands in inducing neck dystonia has been shown to significantly correlate with their binding affinities for the σ receptor labeled with [^{3}H]DTG, a σ_1 and σ_2 receptor agonist (10,27). Similarly, as other ligands with a weak affinity for σ receptors failed to induce neck dystonia (10,27,28), the posture changes are specific to the activation of the σ receptors.

We recently reported a selective σ_1 receptor agonist, 1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl)piperazine dihydrochloride (SA4503) (14). This compound showed a high affinity for the σ_1 receptor subtype, while it had about 100-fold less affinity for the σ_2 receptor subtype, and 36 other receptors, ion channels, and second-messenger systems (14). In addition, SA4503 has σ_1 receptor agonistic properties, because the inhibition curve of SA4503 for [^{3}H](+)-pentazocine binding was shifted to the right in the presence of GTPγS (14). On the contrary, there was no report that showed a selective ligand for the σ_2 receptor subtype. So far only DTG was reported to bind equivalently for both σ and σ_2 receptor subtypes (22). In the present study, to clarify which σ receptor subtypes, namely σ_1 receptor subtype or σ_2 receptor subtype, are related with the σ receptor-mediated neck dystonia, we compared the effects of SA4503, a selective σ_1 receptor agonist with those of DTG, a σ_1 and σ_2 receptor agonist, on the production of neck dystonia in rats.

METHODS

The procedures involving animals and their care were conducted in conformity with institutional guidelines that are in compliance with the "Guide for the Care and Use of Laboratory Animals" (NIH Publication, No. 85-23, 1985).

Animals

Male Wistar rats, weighing 250–350 g at the time of surgery, were used in the following experiments. They were housed three per cage, with free access to food and water, in a controlled environment (23 ± 1°C and 55 ± 10% humidity), with a 12L:12D cycle (light on between 0700 and 1900 h). They were used following at least 7 day's adaptation to laboratory conditions.

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Surgery

On the day of surgery, the rats were anesthetized with sodium pentobarbital (50 mg/kg, IP) and placed in a stereotaxic apparatus. A guide cannula was implanted vertically into the left red nucleus of each animal, according to the Paxinos and Watson (20) atlas of the rat brain (coordinates measured: AP = lambda + 2.9 mm, DV = 8.0 mm, LAT = 0.9 mm, referred to the skull surface). The guide cannula was secured with dental cement and skull screws.

Behavioral Testing

After at least a 24-h recovery time, an injection cannula was inserted into the red nucleus through the guide cannula. The locations of injection cannula probes were confirmed histologically by an examination of brain slice sections.

After microinjection of the test drugs, the rats were photographed every 5 min for 30 min. The neck dystonia was quantified by measuring the torsion of the neck according to the method of Matsumoto et al. (9). Briefly, the torticollis was quantified by measuring the torsional deviation of the head from the horizontal plane, using the eyes of the animals as a reference. Each rat was tested only once to minimize the damage to brain tissue.

Drugs

SA4503 (synthesized in our laboratory) and DTG (Research Biochemicals, Natick, MA) were used. SA4503 and DTG were dissolved in saline on the day of testing. Other chemicals and reagents of an analytical grade were obtained from commercial suppliers. Each rat received a single microinjection of the following compounds: DTG (1, 5, and 10 nmol, $n = 6$, 6, and 9, respectively), SA4503 (1, 5, and 10 nmol, $n = 6$, 6, and 6, respectively), and saline ($n = 6$). Through the injection cannula, drugs were injected in a volume of 0.5 μl over a 1-min period.

Statistical Analysis

Results were expressed as the mean ± SEM. The analysis was carried out using one-way analysis of variance (one-way ANOVA), followed by Dunnett's multiple range comparison test at the same time.

RESULTS

Time-Course Deviation Angle of Neck Dystonia Induced by Microinjection of DTG Into Red Nucleus of Rats

The microinjection of DTG into the red nucleus of the rats induced apparent postural changes characterized by a marked deviation in the head angle (neck dystonia), and later, the contralateral limbs and paws were also affected in some cases. The neck dystonia was significantly induced by 5 nmol/0.5 μl and 10 nmol/0.5 μl DTG (Fig. 1). The statistical values were the following: $F(3, 26) = 1.23$, $p > 0.05$ for 0 min, $F(3, 26) = 2.76$, $p < 0.05$ for 5 min; $F(3, 26) = 4.59$, $p < 0.05$ for 10 min; $F(3, 26) = 2.72$, $p > 0.05$ for 15 min; $F(3, 26) = 5.04$, $p < 0.05$ for 20 min; $F(3, 26) = 3.80$, $p < 0.05$ for 25 min; and $F(3, 26) = 3.10$, $p < 0.05$ for 30 min.

Time-Course Deviation Angle of Neck Dystonia Induced by Microinjection of SA4503 Into Red Nucleus of Rats

The microinjection of SA4503 into the red nucleus of the rats induced little postural change in the head angle. The neck dystonia induced by SA4503 was less than that induced by DTG. In addition, there was no concentration dependency or time dependency at 1 to 10 nmol/0.5 μl injection (Fig. 2). The statistical values were the following $F(3,23) = 1.13$, $p > 0.05$ for 0 min; $F(3, 23) = 1.80$, $p > 0.05$ for 5 min; $F(3,23) = 4.39$, $p < 0.05$ for 10 min; $F(3, 23) = 2.46$, $p > 0.05$ for 15 min; $F(3, 23) = 2.71$, $p > 0.05$ for 20 min, $F(3, 23) = 1.71$, $p > 0.05$ for 25 min; $F(3, 23) = 0.08$, $p > 0.05$ for 30 min.

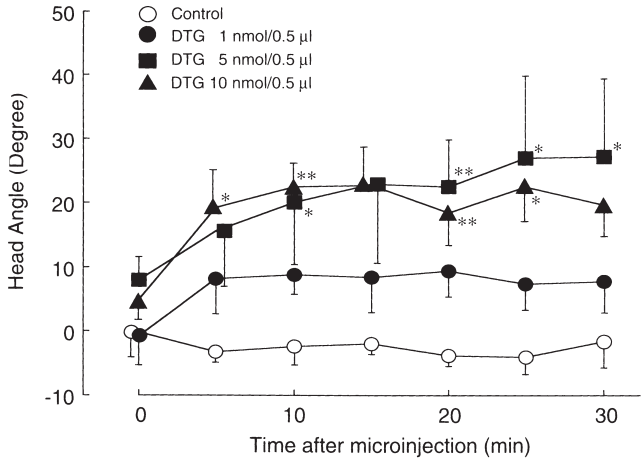

FIG. 1. The neck dystonia induced by microinjection of DTG to red nucleus in rats. Saline (○, $n = 6$), 1 nmol/0.5 μl (●, $n = 6$), 5 nmol/0.5 μl (■, $n = 6$), and 10 nmol/05 μl (▲, $n = 9$) DTG were injected into red nucleus of rats. The data are expressed as means ± SEM. *$p < 0.05$ and **$p < 0.01$ compared to control at the same time.

Maximal Deviation Angle of Neck Dystonia Induced by DTG or SA4503

Maximal deviations of DTG- or SA4503-induced neck dystonia were produced at 25 min after injection and lasted at least until 30 min after injection. The deviation angle of neck dystonia induced by 5 nmol/0.5 μl DTG at 30 min was 27.17 ± 12.33 degrees, which was significantly different from the con-

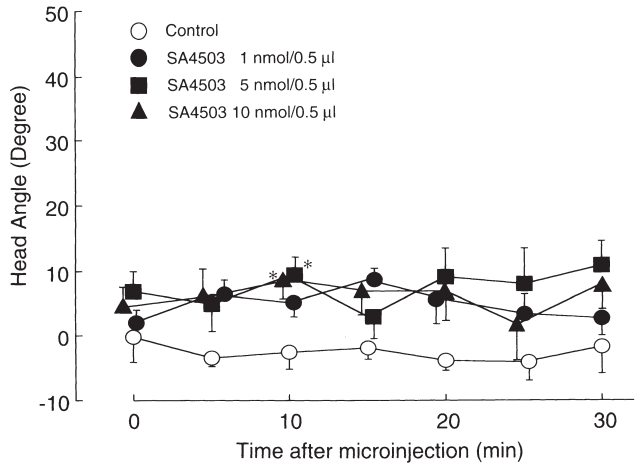

FIG. 2. The neck dystonia induced by microinjection of SA4503 to red nucleus in rats. Saline (○, $n = 6$), 1 nmol/0.5 μl (●, $n = 6$), 5 nmol/0.5 μl (■, $n = 6$), and 10 nmol/05 μl (▲, $n = 6$) SA4503 were injected into red nucleus of rats. The data are expressed as means ± SEM *$p < 0.05$ compared to control at the same time.

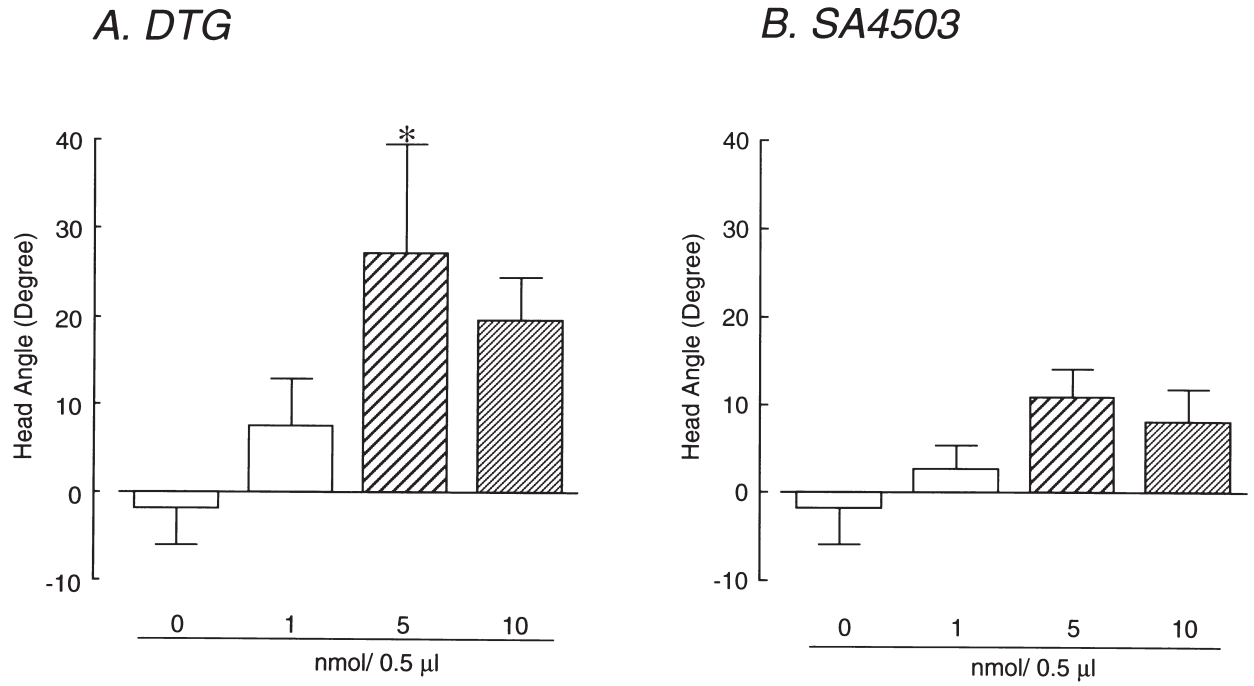

FIG. 3. The deviation angle of neck dystonia induced by (A) DTG and (B) SA4503 at 30 min after microinjection. The data are expressed as means ± SEM of six to nine rats (*$p < 0.05$ compared to control at 30 min after injection).

trol [Fig. 3A, $F(3, 26) = 4.1$, $P < 0.05$]. On the other hand, SA4503-induced neck dystonia was not significant [Fig. 3B, $F(3, 20) = 2.6$, $P > 0.05$].

DISCUSSION

σ Receptors, which were discovered by the pioneering studies of Martin et al. (8), have been considered enigmatic molecular targets. Subsequent intensive studies revealed that there are at least two σ receptor subtypes, called σ_1 and σ_2, which have differences in their binding affinity and physiological functions (21,22). Briefly, the (+)-enantiomer of benzomorphans have a high affinity and selectivity for the σ_1 receptor subtype compared with the (−)-enantiomer of benzomorphans, whereas the opposite stereoselectivity is shown in the σ_2 receptor subtype (4,22). In addition, the σ_1 receptor subtype plays an important role in the facilitation of the central cholinergic function (13,15,17) and the alleviation of depression (11). On the contrary, the σ_2 receptor subtype has been reported to interact with the motor functions (26) and K^+ channels (5). Prototype σ receptor agonists, (+)-SKF-10,047 and (+)-3-PPP, have been reported to show a high degree of selectivity for binding to the σ_1 receptor subtype over the σ_2 receptor subtype (21). However, the selectivity of SA4503 for the σ_1 receptor subtype over the σ_2 receptor subtype was also superior to that of these two prototype σ receptor agonists (14). In addition, the binding affinity of SA4503 for the σ_1 receptor subtype was also superior to that of (+)-SKF-10,047 and (+)-3-PPP. Moreover, (+)-SKF-10,047 has been reported to bind to the *N*-methyl-D-aspartate (NMDA) receptor channel complex (7) and (+)-3-PPP to the dopamine autoreceptor (29). Thus, SA4503 is considered to be a novel, potent, and selective agonist for the σ_1 receptor subtype. With regard to the σ_2 receptor subtype, there is no report that showed a potent and selective agonist and antagonist for this receptor subtype. Only DTG has reported to bind for both the σ_1 and σ_2 receptor subtypes as an agonist (10,21,27). In the present study, we showed that DTG produced neck dystonia, whereas SA4503 did not produce it. These results clearly indicated that the σ_2 receptor subtype plays an important role in the σ receptor ligand-induced neck dystonia in rats, and the σ_1 receptor is scarcely involved in the σ receptor ligand-induced neck dystonia in the rat. These results are coupled to previous demonstrations that the dystonic potency of DTG is greater than that of (+)-pentazocine, a σ_1 receptor agonist (10), and that a potent σ receptor antagonist that has high binding selectivity to the σ_2 receptor subtype reduced haloperidol-induced neck dystonia in rats (9).

Previous investigations showed that the activation of the σ_1 receptor subtype induced antiamnesic, antidepressive, neuroprotective, and cholinomimetic effects in rodents (2,11,12,13, 15,17,23). In addition, we found that SA4503, a novel σ_1 receptor agonist, also induced these effects in both in vivo and in vitro experiments (6,11,16,18,24,25). Thus, σ_1 receptor agonists are expected to be a potent drug for the treatment of disorders with a dementia component and depression. In the present study, we clearly showed that the σ_1 receptor agonist failed to induce neck dystonia in rats. These findings also suggested that σ receptor ligand-induced neck dystonia might be caused by the σ_2 receptor subtype rather than the σ_1 receptor subtype. Coupled to previous demonstrations, σ_1 receptor agonists also failed to induce adverse effects, such as catalepsy, tremor, miosis, and lacrimation mediated by the augmentation of ACh transmission (6,16). Taking all findings into consideration, σ_1 receptor agonists, such as SA4503, may be good candidates for the treatment of CNS disorders.

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