Polish Journal of Pharmacology
Pol. J. Pharmacol., 2001, 53, 547–550
ISSN 1230-6002

PRELIMINARY COMMUNICATION

REPEATED TREATMENT WITH SA4503, A SELECTIVE SIGMA$_1$ RECEPTOR AGONIST, UP-REGULATES α_1-ADRENERGIC SYSTEM. A BEHAVIORAL STUDY

Grażyna Skuza#, Wacław Kolasiewicz

Department of Pharmacology, Institute of Pharmacology, Polish Academy of Sciences,
Smętna 12, PL 31-343 Kraków, Poland

Repeated treatment with SA4503, a selective sigma$_1$ receptor agonist, up-regulates α_1-adrenergic system. A behavioral study. G. SKUZA, W. KOLASIEWICZ. Pol. J. Pharmacol., 2001, 53, 547–550.

The obtained results indicate that SA4503, a selective sigma$_1$ receptor agonist, given repeatedly (but not acutely) enhanced the effects of phenylephrine, α_1-adrenoceptor agonist, and clonidine (stimulating the postsynaptic α_1-adrenoceptors at high dose) in behavioral models (hyperexploratory activity in rats and aggressiveness in mice, respectively).

Key words: *sigma$_1$ agonist, repeated treatment, phenylephrine, clonidine, behavioral models*

SA4503 [1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl)piperazine dihydrochloride] has been described as a new, selective sigma$_1$ (σ_1) receptor agonist with a potential antidepressant activity [8, 9, 17]. It has been reported that SA4503 shows an antidepressant-like activity in mice [8, 17]. Similar effects were observed after the administration of (+)-pentazocine (another σ_1 receptor agonist) and DTG (a σ_1/σ_2 agonist) to mice and rats. Moreover, those antidepressant-like effects were antagonized by the σ_1 receptor antagonist, NE-100 [8, 9, 16, 17].

Our previous studies indicated that SA4503 decreased the immobility time in the forced swimming test in rats and showed a synergistic effect with imipramine in that model. When given repeatedly (14 or 21 days), SA4503 induced effects similar to those produced by many clinically active antidepressant drugs (ADs), e.g. it enhanced the D-amphetamine- or quinpirole-induced hyperactivities in rats, and did not change the D-amphetamine stereotypy (in preparation).

It was demonstrated that ADs administered repeatedly increased the responsiveness of the α_1-adrenergic system (sensitivity of postsynaptic α_1-adrenergic receptors) at both behavioral and biochemical levels (e.g. they increased the clonidine-induced

correspondence

aggressiveness in mice, enhanced the hyperexploratory behavior evoked by the α_1-adrenergic agonists phenylephrine and methoxamine in rats, and increased the binding to α_1-adrenoceptors in the rat cortex) [1, 2, 3, 4, 7, 12]. The above effects have been reported for various ADs (tricyclics, NaSRI, SSRI, MAO inhibitors, mianserin).

The present study aimed to determine whether repeated treatment with SA4503 could induce adaptive changes in the α_1-adrenergic system, similar to those reported by us earlier for several other ADs. To this end, the effects of SA4503 on the response to the compounds stimulating α_1-adrenoceptors were studied in the following tests: phenylephrine-induced hyperexploratory behavior in rats and clonidine-induced aggressiveness in mice.

MATERIALS and METHODS

The experiments were carried out on male Wistar rats (220–230 g) and male Albino Swiss mice (25–30 g). The animals had free access to food and water before the experiment and were kept at a constant room temperature (22 ± 1°C), under a 12/12 h light/dark cycle (light on at 7 a.m.). Experimental protocols were approved by the local Ethics Committee and met guidelines of the responsible agency of the Institute of Pharmacology.

SA4503 [1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl) piperazine dihydrochloride, Santen Pharmaceutical Co., Japan] was dissolved in distilled water and administered *po* once or repeatedly (once daily for 21 days). Control animals received vehicle according to the same schedule. The experiments were carried out 2 h (clonidine-induced aggression) and 2 or 72 h (phenylephrine-induced hyperexploration) after a single (acute treatment) or last dose (repeated treatment) of SA4503. The data were evaluated by an analysis of variance (ANOVA) followed, when appropriate, by individual comparisons with the control using Dunnett's test.

Two hours after the last dose of SA4503 (or after its single administration), clonidine (hydrochloride, Research Biochemicals Int. USA) was injected *ip* at a dose of 20 mg/kg. Immediately thereafter, groups of 4 mice each were placed together in plexiglas cages (20 × 15 cm) and observed for 1 h. Aggression was expressed as the number of biting attacks [13].

For experiments with phenylephrine (hydrochloride, Research Biochemicals Int., USA), the rats were operated under pentobarbital anesthesia (30 mg/kg *ip*) and stainless steel guide cannulae 9.00 mm long (0.4 mm o.d.) were implanted chronically and unilaterally. After a 4-day postoperative period, the animals were treated with SA4503 (or vehicle) for 21 days. Phenylephrine (25 μg /5 μl) was injected into the brain lateral ventricle 30 min before the test, using an inner injection cannula (11.6–14.6 mm long; 0.3 mm o.d.). The tip of injection cannula was aimed at the lateral ventricle (AP (–) 0.4–0.8, L 1.1–1.7, V 3.4) according to appropriate stereotaxic coordinates [14]. The inner cannula was withdrawn 1 min after the termination of the injection (lasting 2 min). Control animals (operated) were treated with appropriate volume of the solvent. Exploratory activity was assessed in the elevated open field test. During the experiment, the laboratory room was dark and only the center of the open field (without walls) was illuminated. The animals were placed in the open field and their exploratory behavior (time of walking, number of sector crossings, episodes of peeping outside the edge of the arena and rearing) was assessed for 5 min. After completion of the experiments, rats were decapitated (under pentobarbital anaesthesia) and perfused through the heart with 4% paraformaldehyde. Then their brains were removed, cut into 50 μm sections and the location of all the injection cannulae tips was determined histologically. Subjects with inappropriate cannula positioning were not included in the analysis. Each group consisted of 8 rats.

RESULTS and DISCUSSION

The obtained results indicated that SA4503 (0.5 mg/kg) given repeatedly (once daily for 21 days), but not acutely, enhanced clonidine-induced aggressiveness in mice (Tab. 1). Repeated treatment with higher dose of SA4503 (3 mg/kg) also increased the effect of clonidine in this test but the difference did not reach the level of statistical significance (Tab. 1).

Similar potentiating effect of repeated treatment with SA4503 was observed in the test with phenylephrine-induced hyperexploration (Tab. 2). Phenylephrine (25 μg/5 μl/rat) given 30 min before the experiment to the operated vehicle-treated rats increased their exploratory behavior in the open field test. SA4503, (3 mg/kg *po*), given repeatedly (but not at a single dose) enhanced the phenylephrine-

-induced hyperexploration, i.e. prolonged the time of walking and increased the number of ambulations as well as rearings and peepings, when measured 2 h after the last dose of SA4503. The potentiating effect of the repeated treatment with SA4503 lasted up to 72 h (at least) after the last administration of SA4503 (Tab. 2).

Table 1. Effect of SA4503 (0.5 and 3 mg/kg, *po*) given acutely or repeatedly on the clonidine (CLO, 20 mg/kg *ip*)-induced aggressive behavior in mice

Treatment	Number of biting attacks (mean ± SEM)	
	Acute	Repeated
Vehicle + CLO	52.5 ± 10.0	44.2 ± 9.7
SA4503 (0.5) + CLO	31.6 ± 7.0	87.4 ± 12.0*
SA4503 (3) + CLO	46.0 ± 10.2	79.0 ± 10.2
ANOVA	F (2, 21) = 1.91 n.s.	F (2, 22) = 4.81 α = 0.05

CLO (20 mg/kg *ip*) was given 2 h after the last dose of SA4503 (0.5 and 3 mg/kg) (repeated treatment), or after a single dose of SA4503 (acute treatment). Immediately after CLO injection, the test was begun, and the number of biting attacks was recorded in 4 mice during 1 h. The results are shown as means ± SEM; n = 8. The obtained data were statistically evaluated by ANOVA, followed by Dunnett's test; * $p < 0.05$ vs. CLO

The effects of SA4503 in the above-described tests are similar to those obtained earlier with repeatedly administered other ADs. Our previous studies have shown that repeated treatment with ADs increases responsiveness of the α_1-adrenergic system (sensitivity of postsynaptic α_1-adrenergic receptors). Potentiation of the behavioral hyperexploration evoked by α_1-adrenergic agonists (phenylephrine, methoxamine), as well as of the clonidine-induced aggressiveness [1, 3, 5, 6] are a measure of α_1-adrenergic system activity, since pro-aggressive effects of clonidine (at a high dose of 20 mg/kg) results from the stimulation of postsynaptic α_1-adrenergic receptors [11]. Moreover, ADs administered repeatedly were shown to increase the binding to α_1-adrenergic receptors in different brain regions, in particular the affinity of these receptors for their agonists [1, 2, 4, 12]. Such an effect was observed with both tricyclic and newer ADs, e.g. venlafaxine or milnacipran [5, 6]. It is worth to add that, in contrast to tricyclic ADs, the latter two drugs, that are the selective noradrenaline and serotonin reuptake inhibitors, show very little (or no) affinity for postsynaptic neurotransmitter receptors, while SA4503

Table 2. Effect of repeated treatment with SA4503 (3 mg/kg, once daily for 21 days) on the phenylephrine (PHEN, 25 µg/5 µl/rat, *ivc*)-induced hyperexploration in rats

Treatment	Mean ± SEM		
	Time of walking	Number of ambulations	Number of rearing + peeping
A.			
Vehicle (21× *po*) + vehicle (*ivc*)	44.3 ± 3.3	17.0 ± 0.8	12.9 ± 0.5
SA4503 (21× *po*) + vehicle (*ivc*)	47.2 ± 6.0	15.8 ± 2.6	13.5 ± 3.9
Vehicle (21× *po*) + PHEN (*ivc*)	76.0 ± 4.6**	20.3 ± 1.1	15.0 ± 3.9
SA4503 (21× *po*) + PHEN (*ivc*)	106.7 ± 3.5##	32.2 ± 3.1#	23.53 ± 2.2#
B.			
Vehicle (21× *po*) + vehicle (*ivc*)	45.8 ± 3.6	16.4 ± 1.4	10.8 ± 1.1
SA4503 (21× *po*) + vehicle (*ivc*)	38.1 ± 5.3	17.0 ± 3.5	12.0 ± 2.4
Vehicle (21× *po*) + PHEN (*ivc*)	68.0 ± 4.5**	27.0 ± 2.0*	14.3 ± 2.9
SA4503 (21× *po*) + PHEN (*ivc*)	91.9 ± 4.9##	36.8 ± 2.6#	17.3 ± 2.0

PHEN was given unilaterally into the cerebral ventricle (*ivc*) at the dose of 25 µg/5 µl, 2 h (A) or 72 h (B) after the last dose of SA4503; 30 min later rats were placed individually in the center of the open field (divided into 6 sectors) and allowed to explore freely for 5 min. Total time of walking, number of sector crossings (ambulations) and sum of numbers of rearings + peepings are expressed as means ± SEM. ANOVA (two-way) followed by Dunnett's test were used for the statistical evaluation of the results. ** $p < 0.001$ vs. vehicle; # $p < 0.05$, ## $p < 0.001$ vs. PHEN

exhibits high affinity only for σ_1 (ca. 100 × higher than for σ_2) and little (or no) affinity for 36 known receptors, ion channels and second messenger systems [9].

These preliminary results suggest that repeated treatment with SA4503, a selective σ_1 receptor agonist, up-regulates the α_1-adrenergic system, similarly as numerous ADs used in the clinic do, though further studies (especially biochemical) are required to support this hypothesis.

It is suggested that the σ_1 receptor is coupled with the cell membrane-bound G proteins, since most physiological effects of σ ligands are sensitive to pertusis toxin [10]. However, the σ_1 receptor differs (structurally and by its subcellular distribution) from conventional membrane-bound receptors known to interact with heterotrimeric G proteins as well as from cytoplasmic proteins/growth factors known to act on monomeric G proteins. This is suggestive of a novel mode of action of intracellular receptors. It is noteworthy that no known mammalian protein is homologous to the cloned σ receptors [15].

In spite of this, the σ_1 receptor agonist SA4503, given repeatedly, induces similar behavioral changes in α_1-adrenergic system as many ADs do but the mechanism involved is not clear as yet and requires further studies.

In conclusion, hitherto existing data on SA4503 appear to indicate that the σ_1 receptor subtype plays an important role in the behavioral responses in depression, and that SA4503 may have an antidepressant activity.

Acknowledgment. The authors wish to thank Dr. K. Matsuno (Santen Pharmaceutical Co., Japan) for generous gift of SA4503.

REFERENCES

1. Maj J.: Antidepressants given repeatedly: pharmacological evaluation of their action. Pol. J. Pharmacol. Pharm., 1991, 43, 241–254.
2. Maj J., Klimek V., Nowak G.: Antidepressant drugs given repeatedly increase binding to α_1-adrenoceptors in the rat cortex. Eur. J. Pharmacol., 1985, 119, 113–116.
3. Maj J., Mogilnicka E., Klimek V., Kordecka-Magiera A: Chronic treatment with antidepressants: potentiation of clonidine-induced aggression in mice via noradrenergic mechanism. J. Neural Transm., 1981, 52, 189–197.
4. Maj J., Przegaliński E., Mogilnicka E.: Hypotheses concerning the mechanism of action of antidepressant drugs. Rev. Physiol. Biochem. Pharmacol., 1984, 100, 1–74.
5. Maj J., Rogóż Z.: Pharmacological effects of venlafaxine, a new antidepressant, given repeatedly, on the α_1-adrenergic, dopamine and serotonin system. J. Neural Transm., 1999, 106, 197–211.
6. Maj J., Rogóż Z., Dlaboga D., Dziedzicka-Wasylewska M.: Pharmacological effects of milnacipran, a new antidepressant, given repeatedly on the α_1-adrenergic and serotonergic 5-HT_{2A} systems. J. Neural Transm., 2000, 107, 1345–1359.
7. Maj J., Rogóż Z., Skuza G.: Antidepressants given repeatedly increase the behavioural effects of methoxamine. Hum. Psychopharmacol., 1989, 4, 65–70.
8. Matsuno K., Kobayashi T., Tanaka M.K., Mita S.: σ_1 Receptor subtype is involved in the relief of behavioral despair in the mouse forced swimming test. Eur. J. Pharmacol., 1996, 312, 267–271.
9. Matsuno K., Nakazawa N., Okamoto K., Kawashima Y., Mita S.: Binding properties of SA4503, a novel and selective σ_1-receptor agonist. Eur. J. Pharmacol., 1996, 306, 271–279.
10. Monnet F.J., Debonnel G., Bergeron R., Gronier B., de Montigny C.: The effects of sigma ligands and of neuropeptide Y on N-methyl-D-aspartate-induced neuronal activation of CA3 dorsal hippocampus neurones are differentially affected by perusis toxin. Brit. J. Pharmacol., 1994, 112, 709–715.
11. Morpurgo C.: Aggressive behavior induced by large doses of 2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride (ST 155) in mice. Eur. J. Pharmacol., 1968, 3, 374–377.
12. Nowak G., Przegaliński E.: Effect of repeated treatment with antidepressant drugs and electroconvulsive shock (ECS) on ^{3}H-prazosin binding to different rat brain structures. J. Neural Transm., 1988, 71, 57–64.
13. Ozawa H., Miyauchi T., Sugawara K.: Potentiating effect of lithium chloride on aggressive behaviour induced in mice by nialamide plus L-DOPA and clonidine. Eur. J. Pharmacol., 1975, 34, 169–179.
14. Paxinos G., Watson C.: The Rat Brain Stereotaxic Coordinates. Academic Press, Sydney, 1986.
15. Seth P., Fei Y.-J., Li H.W., Huang W., Leibach F.H., Ganapathy V.: Cloning and functional characterization of a σ receptor from rat brain. J. Neurochem., 1998, 70, 922–931.
16. Skuza G., Rogóż Z.: Effect of 1,3-di-o-tolylguanidine (DTG), rimcazole and EMD 57445, the σ receptor ligands, in the forced swimming test. Pol. J. Pharmacol., 1997, 49, 329–335.
17. Ukai M., Maeda H., Nanya Y., Kameyama T., Matsuno K.: Beneficial effects of acute and repeated administrations of σ receptor agonists on behavioral despair in mice exposed to tail suspension. Pharmacol. Biochem. Behav., 1998, 61, 247–253.

Received: October 4, 2001; in revised form: October 30, 2001.