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Pharmacological identification of SM-21, the novel σ_2 antagonist

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Abstract

SM-21 is a tropane analogue with high affinity and selectivity for σ_2 receptor subtype. In the absence of highly selective σ_2 antagonists, the aim of the present study was to determine whether SM-21 is endowed with antagonistic activity. The experiments were conducted in rats by inducing neck dystonia, which is reported to be subsequent to activation of σ_2 receptors. SM-21 (10 nmol/0.5 μl) was able to prevent torsion of the neck obtained by administration of the $\sigma_1-\sigma_2$ agonist 1,3-di-(2-tolyl)guanidine (DTG, 5 nmol/0.5 μl) in the red nucleus. These data indicate that SM-21 is a potent and selective σ_2 antagonist.

Keywords: SM-21; σ_2 receptor subtype; Neck dystonia; Red nucleus; DTG; AC927

1. Introduction

The σ binding sites were first postulated in 1976, as a subtype of opiate receptor [10]. They are widely distributed throughout the brain and periphery, leading to speculation that the σ-mediated system may play a role in psychosis [22], motor dysfunction [22], emetic processes [8] and hepatic [22], endocrine [22] and intestinal functions [1]. However, relatively little is actually known about the functional significance of these binding sites.

The existence of at least two subtypes of σ binding sites, classified as σ_1 and σ_2, has been reported by Hellewell and Bowen [7]. It was shown that σ_1 and σ_2 sites exist in both brain and periphery in a wide variety of species [21,24], although with different anatomical distribution [7]. They also occur as proteins with different molecular weights (approximately 25 and 18–21 kDa, respectively) [7]. In addition to these anatomical differences, σ_1 and σ_2 receptors have different physiological functions. σ_1 receptor subtype induces antiamnesic, antidepressive, neuroprotective and cholinomimetic effects in rodents [2,13,14,19], whereas σ_2 receptors have been reported to interact with the motor functions, such as circling and acute dystonic reactions [23].

The tropane analogue 3-α-tropanyl-2(*p*Cl-phenoxy) butyrate, labeled SM-21, is an ACh releaser endowed with antinociceptive and antiamnesic properties [4]. Recent binding studies evidenced that SM-21 is also a selective ligand for σ_2 receptors [9], but its agonist/antagonist nature at σ_2 receptors has not been established.

2. Methods

2.1. Subjects

Male Wistar rats weighing 250–350 g were used. They are housed three per cage, with free access to food and water, in a controlled environment (23 ± 1°C and 55 ± 10% humidity), with 12L/12D cycle. They were used following at least 7 days adaptation to laboratory conditions. The procedures involving animals and their care were conducted in conformity with institutional guidelines that comply with the "Guide for the Care and Use of Laboratory Animals" (NIH Publication No. 80-23, Revised 1978).

2.2. Apparatus

The neck dystonia was quantified by measuring the torsion of the neck according to the method of Matsumoto et al. [11]. 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.

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Each rat was tested only once to minimize the damage to brain tissue.

2.3. Procedure

The rats were anaesthetized with sodium pentobarbital (50 mg kg^{-1}, ip) and placed in a stereotaxic apparatus. A guide cannula was implanted vertically into the left red nucleus of each animal, according to Paxinos and Watson's [18] atlas of the rat brain (coordinates measured: AP = lamba + 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.

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. Animals received a single microinjection of the following compounds: 1,3-di-(2-tolyl)guanidine (DTG, Research Biochemicals), SM-21 (prepared at the Department of Pharmaceutical Sciences of the University of Florence, according to Gualtieri et al. [5]) and AC927 (phenethylpiperidine). Through the injection cannula, drugs were injected in a volume of 0.5 μl over a 1-min period.

2.4. Statistical analysis

All data were presented as mean ± S.E.M.; 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. $P < .05$ was considered significant.

3. Results

The microinjection of DTG (5 nmol/0.5 μl) into the red nucleus of the rats induced postural changes characterized by a marked deviation in the head angle (neck dystonia) (Fig. 1). Maximal deviation of DTG was produced at 25 min after microinjection and lasted at least until 30 min after administration (Fig. 1). The σ_2 antagonist AC927 (66 nmol/0.5 μl) prevented the DTG-induced neck dystonia and moved the head angle degree back to a value comparable to that obtained in the control group (data not shown). SM-21 (10 nmol/0.5 μl) was able to completely antagonize the DTG-induced deviation in the rat head angle (Fig. 1). SM-21, administered 5 min before DTG, exerted its antagonistic effect at all observation times (Fig. 1). A dose of SM-21 three times lower than 10 nmol/0.5 μl was unable to reduce the neck dystonia induced by DTG (data not shown).

SM-21, when given alone at the same dose, did not produce any postural change after microinjection in the rat red nucleus (Fig. 1). Furthermore, at the active concentration, it did not modify either the rat's gross behavior or induce any side effect (data not shown).

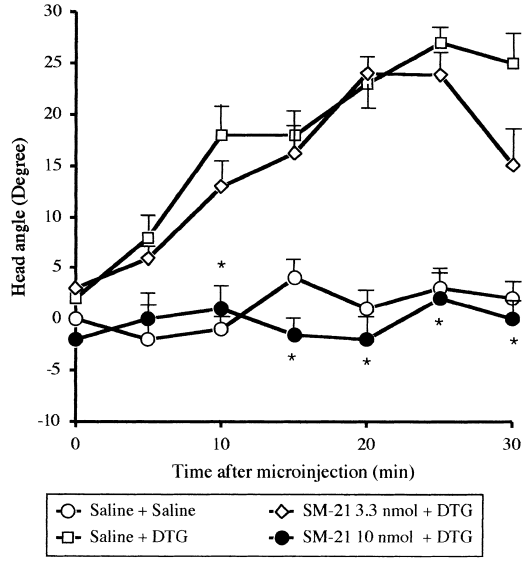

Fig. 1. Dose–response curve of SM-21 on neck dystonia induced by DTG (5 nmol) in the rat red nucleus. SM-21 was administered 5 min before DTG. Data are presented as mean ± S.E.M. All doses are solubilized in 0.5 μl. Each point represents the mean of five to seven rats. * $P < .01$ compared to DTG-treated rats at the same time.

4. Discussion

Present results indicate that the tropane analogue SM-21, which was identified as a selective ligand for σ_2 receptors [9], is endowed with antagonistic properties.

It has been reported by behavioral and biochemical studies that σ receptors are involved in the regulation of movements and posture stems. In particular, σ receptors are concentrated in brain structures that control movement, such as the red nucleus and substantia nigra [6]. The unilateral microinjection of σ receptor ligands, such as DTG, (+)-*N*-allylnormetazocyne [(+)-SKF-10,047] and (+)-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine [(+)-3-PPP], into the red nucleus induces neck dystonia in rats [12,16,22]. 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 the σ_1 and σ_2 agonist [^{3}H]DTG [12,22]. Similarly, as other ligands with a weak affinity for σ receptors, they failed to induce neck dystonia [12,22]. The posture changes are specific to the activation of the σ receptors.

The antidystonic effect in rats was observed to be related to σ receptor antagonism [11], and, in particular, the σ receptor responsible for inducing this postural effect belongs to the σ_2 subtype [16]. The administration of DTG, that was reported to bind equivalently for both σ_1 and σ_2 subtypes, induced a marked deviation in the head angle, which was prevented by AC927, the only available σ_2 antagonist [20],

whereas the σ_1 agonist SA4503 was unable to produce rat postural changes [16]. Therefore, we investigated the σ_2 antagonistic properties of SM-21 by inducing rat neck dystonia, since this animal model represents a suitable in vivo experimental test to selectively reveal σ_2 ligands.

SM-21, being able to prevent a postural effect mediated by activation of σ_2 receptors, represents a useful pharmacological tool for investigating the physiological role of σ_2 receptor subtype. Radioligand binding studies showed that SM-21 had a higher affinity for σ receptors than other receptors, such as opiate, muscarinic, dopamine, serotonin, α-adrenergic (higher than 10^{-6} M) [4,9]. Furthermore, SM-21 was able to discriminate well between σ_1 and σ_2 subtypes, exhibiting a marked higher affinity for σ_2 (67 nM) [9]. On the basis of this binding profile, SM-21 permits the selective study of σ_2-mediated effects also in experimental models where σ_1 and σ_2 receptor subtypes coexist.

The tropane derivative SM-21 is endowed with analgesic and antiamnesic properties through an increase of the ACh release [4]. However, we can exclude the involvement of the cholinergic system into the antidystonic activity of SM-21, since it has been reported that cholinesterase inhibitors induce dystonic-like reaction [15], whereas botulinum toxin gives relief to patients with cervical dystonia by causing a presynaptic block of ACh release [3,17].

Activation of σ_2 receptors induces a rise in intracellular calcium ($[Ca^{2+}]_i$) levels and causes apoptotic cell death [20]. SM-21, being endowed with σ_2 antagonistic properties, could represent a new anticytotoxic agent useful in neurodegenerative disorders induced by apoptotic mechanisms. SM-21 has been reported to be able to prevent amnesia induced by exposure to a hypoxic environment [4], a condition that produces impairment of cognitive processes through apoptotic cell death.

Present results indicate that the tropane analogue SM-21 acts as antagonist at σ_2 binding sites. These antagonistic properties have been revealed by inducing neck dystonia in rats, a behavioral test suitable to identify σ_2 agonists and antagonists. SM-21 was able to prevent the head deviation induced by σ_2 receptor activation (DTG) indicating that it is endowed with antagonistic properties. This compound also represents the most potent full σ_2 antagonist so far available.

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