Further evidence for an antidepressant potential of the selective $\sigma_1$ agonist SA4503: electrophysiological, morphological and behavioural studies

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Abstract

In this study, we evaluated the ability of the selective $\sigma_1$ agonist SA4503 to produce changes in brain function, similar to those elicited by classical antidepressants. We focused more specifically on the influence of SA4503 on central serotonergic (5-HT) transmission, and on hippocampal cell proliferation. A 2-d continuous treatment with SA4503 (1–40 mg/kg·d) increased 5-HT neuron firing rate in a dose-dependent, bell-shaped manner, with a culminating effect of $+90\%$ at 10 mg/kg·d. The same dose induced the appearance of a 5-HT$_{1A}$ receptor-mediated inhibitory tonus on hippocampal pyramidal neurons, as revealed by intravenous injections of the selective 5-HT$_{1A}$ antagonist WAY 100635. Moreover, continuous administration of SA4503 (3 and 10 mg/kg·d, 3 d) dose-dependently enhanced the number of bromodeoxyuridine-positive cells in the subgranular zone of the hippocampus ($+48\%$ and $+94\%$, respectively), thus indicating an increased cell proliferation. Finally, a single administration of SA4503 (3 and 10 mg/kg i.p.) increased the time spent swimming in the forced swimming test. Together, these results provide both functional and behavioural evidence that this compound has an important antidepressant potential. Further, the fact that the functional changes occurred within a short time-frame (2–3 d) suggest that this antidepressant potential might have a rapid onset of action.

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Introduction

During the last two decades, a growing number of studies led to the identification of various biochemical, physiological or anatomical markers of antidepressant (AD) action within the brain. Among these, the level of central serotonergic (5-HT) transmission has been extensively studied. It is now accepted that, despite the variety of their pharmacological profiles and primary targets, a common trait of all AD molecules resides in their ability to increase this parameter (Blier and de Montigny, 1994, 1999; Michelsen et al., 2007; Szabo and Blier, 2001). More specifically, these compounds are known to enhance the tonus exerted on post-synaptic 5-HT$_{1A}$ receptors within limbic areas such as the hippocampus (Blier and Ward, 2003; Haddjeri et al., 1998; Szabo and Blier, 2001). It has also more recently been shown that AD administration facilitates adult neurogenesis in this same brain region, an effect that seems to be causally related to the beneficial effect of the treatment (Malberg et al., 2000; Santarelli et al., 2003; Scharfman and Hen, 2007). In parallel to these functional studies, several behavioural tests have been developed in rodents in an attempt to establish models, predictive of an AD potential for a given molecule. One of the most reliable is the forced swimming test (FST), in which the animal is placed in a cylinder of water from which it cannot escape, and subsequently develops

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an immobile posture thought to reflect ‘behavioural despair’ (Cryan et al., 2002). Thus far, all clinically used AD have proved to be efficient in reducing the duration of this immobility period (Cryan et al., 2002).

Sigma (σ) binding sites, first identified serendipitously in 1976 (Martin et al., 1976), are now considered as constituting a distinct family of receptors, and have been subdivided into the σ₁ and σ₂ types (Quirion et al., 1992). Interestingly, it appears that σ₁ ligands act similarly to classical AD in the FST (Oshiro et al., 2000; Urani et al., 2001, 2004). In addition, several σ₁ agonists have been shown to enhance 5-HT neuron firing rate after both short-term (2 d) and long-term (21 d) chronic administration (Bermack and Debonnel, 2001), suggesting that such treatments may result in an enhancement of central 5-HT neurotransmission. Moreover, a few studies conducted on cultured cell lines indicate that the stimulation of σ₁ receptors facilitates cellular processes related to neurogenesis, such as neurite sprouting or axon outgrowth and guidance (Takebayashi et al., 2002, 2004). Altogether, these findings suggest that σ₁ receptor agonists may have the potential for the treatment of depression. Among the agonists that have been developed in the past few years, l-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl)piperazine dihydrochloride (SA 4503 dihydrochloride) appears to be of particular interest. Indeed, this compound has been shown to have a >100-fold selectivity for σ₁ binding sites over σ₂ ones, and a very low affinity for a wide range of neurotransmitter receptors and ion channels (Matsumo et al., 1996a; Senda et al., 1996). SA 4503 is able to cross the blood–brain barrier and to exert various central effects that are antagonized by selective σ₁ receptor blockade (Kobayashi et al., 1997; Senda et al., 1996; Ukai et al., 1998; Zou et al., 2000). Further, previous behavioural studies have already shown that SA 4503 is effective in the FST in both mice and rats (Matsumo et al., 1996b; Skuza and Rogóz, 2002).

This study was aimed at evaluating the AD potential of SA 4503 in a more complete and systematic manner. In particular, given the key role played by 5-HT in the mechanism of action of AD (see above), the average activity of 5-HT neurons was measured in vivo within the rat dorsal raphe nucleus (DRN), from which most of the central 5-HT innervation originates (Michelsen et al., 2007), after 2 d of continuous SA 4503 administration. Because σ₁ agonists are typically characterized by a bell-shaped dose–response curve in various experimental paradigms (Bergeron et al., 1995; Hayashi et al., 2000; Maurice et al., 1994a,b; Monnet et al., 1996), these experiments were conducted with a wide range of SA 4503 doses (1–40 mg/kg). Thereafter, the optimal dose was tested on the tonus exerted by endogenous 5-HT on hippocampal post-synaptic 5-HT₁A receptors, following an experimental protocol designed in our laboratory (Haddjeri et al., 1998). In addition, we also assessed the effect of SA 4503 on adult brain cell proliferation by measuring the accumulation of bromodeoxyuridine (BrdU) in subgranular cells of the hippocampus (Malberg et al., 2000). Finally, SA 4503 was tested in the FST, to further confirm both the AD potential of the drug, and previous results obtained in rats with this compound (Skuza and Rogóz, 2002).

Materials and methods

Animals

Experiments were carried out using male Sprague–Dawley rats (Charles River, St Constant, Québec, Canada), weighing 250–300 g and which were kept under standard laboratory conditions [12 h light–dark cycle (lights on 07:00 hours) with free access to food and water]. All animals were handled according to the guidelines approved by the Faculty Ethical Committee of McGill University.

Drugs and chemicals

The following compounds were used: SA 4503 dihydrochloride (provided by M’s Science Corporation, Kobe, Japan), WAY 100635 hydrochloride (Research Biochemicals, Natick, MA, USA), BrdU (Sigma-Aldrich Canada, Oakville, ON, Canada). SA 4503 and WAY 100635 were diluted in distilled water, and BrdU in Tris-buffered saline (0.1 M in NaCl 0.9%, pH 7.6). Drug dosages refer to the free base, except for SA 4503 (referring to the salt). In electrophysiological and morphological experiments, animals underwent a chronic short-term (2 d or 3 d) treatment with SA 4503. The drug was continuously administered by using osmotic minipumps (model: Alzet 2ML1, 2 ml reservoir, 10 μl/h delivery rate; Palo Alto, CA, USA), inserted subcutaneously in the back region under short-duration (≤5 min) halothane anaesthesia. In the case of morphological experiments (assessment of mitogenesis), rats also received a total of six injections of BrdU. These treatments consisted of two administrations per day (50 mg/kg i.p. each), separated by intervals of 8 h, starting from the day of minipump insertion until day 2 post-surgery. Animals were then sacrificed on day 3 post-surgery, 18 h after the last BrdU administration.
**Extracellular recordings of DRN 5-HT neurons**

Recordings were performed using single-barrelled glass micropipettes. Electrodes were filled with a 2 M NaCl solution saturated with Fast Green FCF, resulting in an impedance of 2–5 MΩ. Rats were anaesthetized with chloral hydrate (400 mg/kg i.p.) and placed in a stereotaxic frame. A burr hole was drilled on the midline 1 mm anterior to lambda. DRN 5-HT neurons were encountered over a distance of 1 mm starting immediately below the ventral border of the Sylvius aqueduct. These neurons were identified using the classical criteria: a slow (0.5–2.5 Hz) and regular firing rate and long-duration (0.8–1.2 ms) positive action potentials (Aghajanian, 1978). At the end of the experiments, a 25 μA cathodal current was passed through the recording electrode to leave a Fast Green deposit at the recording site. Animals were sacrificed with an i.v. overdose of chloral hydrate, and the brain removed. The site of recording was verified under microscope immediately after experiments.

**Extracellular recordings from hippocampal CA3 pyramidal neurons**

Recording were performed with five-barrelled glass micropipettes broken back to 8–12 μm under microscope control. The central barrel was filled with the same solution as in the DRN and was used for extracellular unitary recordings. Pyramidal neurons were identified by their large amplitude (0.5–1.2 mV) and long-duration (0.8–1.2 ms) simple spikes alternating with complex spike discharges (Kandel and Spencer, 1961). The side barrels contained the following solutions: quisqualate (1.5 mM in 200 mM NaCl, pH 8) and 2 mM NaCl used for automatic current balancing. Rats were mounted in the stereotaxic apparatus and the micropipettes were lowered at 4.2 mm lateral and 4.2 mm anterior to lambda into the CA3 subregion of the dorsal hippocampus. A catheter was inserted in a lateral vein of the tail, in order to assess the acute effect of the 5-HT1A antagonist WAY 100635.

**Measurement of hippocampal cell proliferation**

Animals were deeply anaesthetized with an overdose of sodium pentobarbital (75 mg/kg i.p.), and perfused transcardially with an initial wash of heparinized 0.9% saline (50–100 ml, 4 °C), followed by 4% paraformaldehyde in phosphate buffer (300 ml, 0.1 M, pH 7.4, 4 °C). Brains were immersed for 48 h in 30% phosphate-buffered sucrose solution (pH 7.4) and then cut in the coronal plane at 50 μm on a sliding freezing microtome. Free-floating sections were collected in phosphate-buffered saline (PBS, 0.1 M, pH 7.4) as separate sets so that each set contained every sixth serial section. Selected adjacent free-floating sections were processed for double-labelling immunohistochemistry for BrdU and Nissl staining, by using minor modifications of a previously published method (Sadikot and Sasseville, 1997; Soriano and Del Rio, 1991). Briefly, sections were incubated in 0.5% sodium borohydride dissolved in PBS for 20 min and rinsed twice in PBS. They were then incubated for 30 min in 1% Triton X-100 in PBS containing 0.03% hydrogen peroxide, followed by 1% dimethylsulfoxide (DMSO) in PBS for 10 min. Sections were immersed in 2 N HCl in PBS for 60 min, and then neutralized by rinsing in sodium borate buffer (0.1 M, pH 8.5) for 5 min. After brief washes in PBS (3 × 5 min each), they were pre-incubated in PBS containing 10% bovine serum albumin (BSA) and 0.3% Triton X-100 for 30 min, briefly rinsed in PBS, and then incubated for 14–16 h in PBS containing anti-BrdU antibody (1:40, Becton Dickinson, San Jose, CA, USA) and 2% BSA (4 °C). After three brief rinses in PBS, sections were incubated in PBS containing secondary antibody (biotinylated anti-mouse IgG, 1:200, Vector, Burlingame, CA, USA) and 2% BSA. Following three brief rinses in PBS, sections were incubated for 1 h in avidin-biotin complex (ABC, 1%, in PBS, Vector). Next, sections were briefly rinsed three times in PBS, and the immunohistochemical reaction product was revealed by incubating for 7–10 min in a solution containing 0.37 mg nickel ammonium sulphate, 25 mg 3,3'-diaminobenzidine tetrahydrochloride (DAB), and 2 μl hydrogen peroxide (30%) dissolved in 100 ml Tris buffer (0.05 M, pH 7.6). This nickel-enhanced DAB-based chromogen yields a blue-black reaction product. Sections were thoroughly rinsed in PBS, and then mounted out of distilled water on glass slides, air-dried, dehydrated in a 80% ethanol solution overnight, then proceed with Cresyl Violet (CV) staining, cleared in Xylene Substitute (Shandon, Pittsburgh, PA, USA), and cover-slipped with Permount (Fisher, Fair Lawn, NJ, USA).

BrdU/CV double-labelled neurons were analysed throughout the entire extent of the granular cell layer (GCL) of dentate gyrus including the subgranular zone (SGZ; as defined by a two-cell-body wide zone at the edge of GCL) at one comparable coronal section (equivalent to Bregma −4.52 mm) (Paxinos and Watson, 1986) in SA 4503- (10 mg/kg), SA 4503- (3 mg/kg) and vehicle-treated animals (n = 4 in each group). The number of BrdU-positive neurons of treated and control animals were compared throughout the cross-section area of interest, and results were expressed both in absolute values and as a
density per mm². Dentate gyrus distribution of BrdU-positive neurons was plotted using a system for image analysis. The left hemisphere was used for all quantitative analysis. The system consisted of a light microscope (BX40, Olympus, Japan) equipped with an X–Y movement-sensitive stage (BioPoint XYZ, LEP, Hawthorne, NY, USA), a Z-axis indicator (MT12 microcator, Heidenhain, Traunreut, Germany) and a video camera (DC200, DAGE, Michigan City, IN, USA) coupled to a computer containing software for computer-assisted image analysis (Stereo Investigator, Microbrightfield Inc., Colchester, VT, USA). The software allowed drawing of outlines of hippocampus sections at low magnification (10 × objective), and plotting of positions of single-labelled (CV) or double-labelled (BrdU/CV) neurons evaluated at high magnification (100 × objective).

**FST**

The FST was performed by using a two-session procedure modification of the protocol originally described by Porsolt et al. (1977). Briefly, rats experienced a pre-test session followed 24 h later by a test session. For both the pre-test and the test sessions, conducted under low illumination (15 W), the animals were placed in a plastic cylindrical tank (50 cm high × 20 cm diameter) filled with water at 24 ± 1°C, with a depth of 40 cm, at which the hind limbs could not reach the tank floor. In all experiments, the pre-test was carried out for 15 min and the test for 5 min in the same tank but the last 4 min only were analysed. SA 4503 (3 or 10 mg/kg) was administered i.p. 30 min before the test session. Following either pre-test or test sessions, rats were dried with a towel and kept warm for 30 min before returning to their home cage. A camera coupled with a computer recorded online animal behaviour during the FST through a specialized digital interface (Videotrack, ViewPoint, Lyon, France). This interface underscored online the subtraction of video frames. Immobility time in the FST was derived from the number of frames (every 40 ms) being below a predefined threshold over FST duration. This threshold was preliminarily set up in order to obtain about 95% of the corresponding frames classified as immobile for a non-climbing, non-swimming rat in its water tank. The same threshold was kept constant for naive as well as treated animals. A virtual rectangle was also defined through the use of the interface, corresponding to the space between the surface of water and the upper limit of the cylindrical tank. Climbing behaviour was counted as the time spent by the animal in this area. Hence, for each animal, the addition of immobility time plus climbing time plus swimming time gives a result of 240 s (i.e. the 4 min period of analysis).

**Statistical analysis**

All statistics were performed by using one-way ANOVAs, followed by Dunnett’s or Tukey’s tests when multiple comparisons were necessary.

**Results**

**Dose–response effect of a 2-d treatment with SA 4503 on DRN 5-HT neuron firing rate**

In agreement with previous reports from our laboratory (Lucas et al., 2005), basal firing activity of DRN 5-HT neurons in control (vehicle) animals was in the 1–1.5 Hz range, with a mean value of 1.25 ± 0.1 Hz. As shown in Figure 1a, SA 4503 (1, 3, 5, 10 and 40 mg/kg, d) dose-dependently augmented this activity when chronically administered for 2 d [one-way ANOVA: F(4, 269) = 2.6, p < 0.05]. The effects of the doses of 1 mg/kg (+18% above basal values) and 3 mg/kg (+30%) were not significant, but those of 5 mg/kg (+51%) and 10 mg/kg (+90%) reached statistical significance (Dunnett’s test: p < 0.05 and p < 0.01 vs. vehicle, respectively). Figure 1b illustrates two samples of tracks (integrated firing rate histograms) performed along the DRN, one in a control rat and the other one after 2 d of SA 4503 at a dose of 10 mg/kg. Whereas, as mentioned above, most 5-HT neurons fired at around 1–1.5 Hz in vehicle-treated animals, it was not uncommon to observe frequencies as high as 3 Hz or even 4 Hz in the SA 4503 group. Interestingly, administration of 40 mg/kg SA 4503 remained without any influence on the 5-HT neuron firing rate, as the values measured in this group were remarkably identical to those found in the vehicle group (1.25 Hz, Dunnett’s test, n.s.). The dose–response curve of SA 4503 on 5-HT neuron firing activity appeared therefore to display a biphasic, inverted U (bell) shape, as previously observed with 5-HT receptor agonists in a number of behavioural, biochemical or other electrophysiological paradigms (Bergeron et al., 1995; Hayashi et al., 2000; Maurice et al., 1994a, b; Monnet et al., 1996).

**Effect of 2-d treatment with 10 mg/kg SA 4503 on the response of hippocampal pyramidal neurons to a 5-HT₄A antagonist**

In a second step of experiments, the firing rate of pyramidal neurons was recorded in the CA3 subfield of the hippocampus. Because most hippocampal
Pyramidal neurons are not spontaneously active under chloral hydrate anaesthesia, a leak or a small ejection current (0 to \( \pm 2 \) nA) of quisqualate (through the use of a multi-barrelled electrode and microiontophoretic pumps) is necessary to activate them within their physiological firing range of 10–15 Hz (Haddjeri and Blier, 1995; Haddjeri et al., 1998). However, in this case the ejection current was adjusted (0 to \( \pm 1 \) nA) so that pyramidal neuron activity reached only 3–6 Hz, and cumulative doses of the selective 5-HT\(_{1A}\) antagonist WAY100635 were administered thereafter. As reported in previous studies from our laboratory (Besson et al., 2000; Blier and Ward, 2003; Haddjeri et al., 1998), this paradigm allows the revelation of an increased tonic stimulation of inhibitory post-synaptic 5-HT\(_{1A}\) receptors. Thus far, all clinically effective AD treatments have been shown to induce the appearance of such a tonus (Besson et al., 2000; Haddjeri et al., 1998). As illustrated in Figure 2, there was virtually no effect of WAY100635 (cumulative doses, 100–500 \( \mu \)g/kg i.v.) in control animals, in agreement with earlier findings reporting that there is no 5-HT\(_{1A}\) tone in this brain area in naive, anaesthetized animals (Besson et al., 2000; Haddjeri et al., 1998). In contrast, WAY100635 had a clear excitatory action in rats receiving chronic treatment of SA 4503 (10 mg/kg . d) for 2 d. Pyramidal neuron activity increased to 140% of basal value after the injection of 100 \( \mu \)g/kg of WAY100635, this augmentation being more prominent and reaching a plateau starting from 200 \( \mu \)g/kg (168, 178 and 167% of baseline at 200, 300 and 500 \( \mu \)g/kg, respectively). Thus, the facilitation of 5-HT neuron firing rate induced by 2-d treatment with 10 mg/kg SA 4503 translated into the appearance of a 5-HT\(_{1A}\)-mediated tonic inhibitory effect on CA3 pyramidal neurons.
Effect of 3-d treatment with 10 mg/kg . d SA 4503 on hippocampal cell proliferation

It is now well established that classical AD promote adult cell proliferation in the hippocampus, an effect occurring predominantly in the SGZ of the dentate gyrus (Malberg et al., 2000; Nakagawa et al., 2002; Santarelli et al., 2003). The ability of SA 4503 to induce such a phenomenon was therefore tested, both at the optimal dose of 10 mg/kg . d, and at the sub-maximal one of 3 mg/kg . d. Figure 3a shows that SA 4503 was able to significantly enhance the number of BrdU-positive cells in the SGZ, in a dose-dependent manner \[ \text{one-way ANOVA: } F(2, 11) = 10.01, \ p < 0.01 \]. In close parallel with the electrophysiological results obtained in the DRN, the augmentation induced by the dose of 10 mg/kg . d \(+94\%\) was statistically significant (Dunnett’s test: \( p < 0.01 \) vs. vehicle), whereas the effect of 3 mg/kg . d \(+48\%\) failed to reach significance (Dunnett’s test, n.s.). Similar results were found when cell proliferation was expressed as a density of BrdU-positive cells/mm\(^2\) \[ \text{one-way ANOVA: } F(2, 11) = 11.8, \ p < 0.01; \text{ Figure 3b} \]. However, in this case, the effect of both doses was statistically significant (\( p < 0.05 \) and \( p < 0.01 \) vs. vehicle for 3 and 10 mg/kg . d, respectively, Dunnett’s test). A detailed examination of hippocampal sections indicated that, in either vehicle- or SA 4503-treated animals, BrdU-positive cells were clearly restricted to the SGZ of the dentate gyrus, at the border between the granular layer and hilus (Figure 4). This distribution is in very good agreement with previous morphological studies, performed using classical AD (Malberg et al., 2000).

Effects of SA 4503 in the FST

As shown in Figure 5a, SA 4503 dose-dependently reduced the time of immobility in the FST \[ \text{one-way ANOVA: } F(2, 23) = 4.52, \ p < 0.05 \]. This parameter was reduced by 25% and 42% with respect to control (vehicle) values after the administration of 3 and 10 mg/kg of the drug, respectively, and only the reduction induced by the higher dose reached statistical significance (\( p < 0.01 \) vs. vehicle, Dunnett’s test). This effect was closely related to an increase in the time spent swimming, as illustrated in Figure 5b \[ \text{one-way ANOVA: } F(2, 23) = 3.85, \ p < 0.05 \]. Once again, the augmentation observed at a dose of 10 mg/kg \(+25\%\) was statistically significant (\( p < 0.05 \) vs. vehicle, Dunnett’s test), whereas that elicited by 3 mg/kg was not. In contrast, SA 4503 had no effect on climbing behaviour \[ \text{one-way ANOVA: } F(2, 23) = 0.6, \text{ n.s.} \], even though a small, non-significant increase was observed after the injection of the lower dose (Figure 5b).

Discussion

The present data confirm and further extend the results of previous studies (Matsuno et al., 1996b; Ukai et al., 1998; Skuza and Rogóz, 2002), suggesting that the selective \( \alpha_1 \) agonist SA 4503 has a potential as an AD drug. A short-term (2–3 d) continuous treatment with this compound increased central 5-HT neurotransmission, as well as cell proliferation within the adult hippocampus. Moreover, a single, subacute injection of SA 4503 was seen to be effective in the FST.
In agreement with earlier reports from our laboratory, and performed with other $\sigma_1$ receptor agonists (Bermack and Debonnel, 2001), a 2-d treatment with SA 4503 resulted in an enhancement of DRN 5-HT neuron firing rate. The effect was dose-dependent, and displayed a bell-shaped dose–response curve. A similar curve has also been reported concerning the modulatory role of $\sigma_1$ agonists on the facilitation of pyramidal neuron electrical activity, and of noradrenalin release, induced by the glutamate analogue NMDA in the hippocampus (Bergeron et al., 1995; Monnet et al., 1996). More generally, biphasic dose–response curves were observed when assessing the effect of several $\sigma_1$ agonists in a variety of experimental paradigms, ranging from behavioural, memory tests in mice (Maurice et al., 1994a, b) to the study of intracellular Ca$^{2+}$ regulation in cultured cell lines (Hayashi et al., 2000). To our knowledge, this is the first time that this property has been shown to apply to the in-vivo firing rate of DRN 5-HT neurons. Considering both the diversity of $\sigma_1$ agonists with which it occurs, and the growing number of experimental models in which it has been observed, the ‘bell-shaped modulation’ appears to constitute a general feature of $\sigma_1$ neurotransmission. Interestingly, cellular transduction mechanisms consequent to $\sigma_1$ receptor stimulation have been found to be multiple and complex, the receptor itself being able to regulate the function of multiple ion channels at both the

Figure 4. Photomicrographs (magnification: $\times$10 and $\times$100 for the small rectangles) representative of the histograms shown in Figure 3. (a) BrdU-positive cells in a vehicle-treated animal; (b) a rat administered 10 mg/kg of SA 4503 (3 d). Note the clear delineation of BrdU-positive cells, restricted to the subgranular zone of the dentate gyrus (border between the granular layer and hilus).

Figure 5. (a) Effect of SA 4503 (3 and 10 mg/kg i.p.) on the time spent immobile in the forced swimming test. All data are expressed as mean ± S.E.M. of eight animals per group, and are from an observation of 4 min duration. Rats experienced a pre-test session (15 min) 24 h before the test session. SA 4503 or its vehicle was administered in a single bolus, 30 min before the test session. (b, c) Effect of SA 4503 on the time spent swimming and climbing, respectively, in the same animals as in panel (a) (* $p<0.05$ and ** $p<0.01$ vs. vehicle, Dunnett’s test).
plasma membrane and the endoplasmic reticulum levels (for review see Bermack and Debonnel, 2005). That ρ-binding sites are able to interact beyond the plasma membrane with several subcellular compartments is not surprising, if one considers the converging data suggesting that their endogenous ligand(s) may be of neurosteroidal nature (Bermack and Debonnel, 2005). It could therefore be proposed that different extracellular concentrations of ρ1 ligands, either endogenous or exogenous, might result in different ‘driving forces’ for the subcellular repartition of the ligand–receptor complex, which in turn would result in distinct, opposite cellular responses. Alternatively, the existence of several subtypes of the ρ1 receptor, exerting antagonistic influences on transduction systems and for which ρ1 ligands may have distinct affinities, has also been hypothesized to explain the biphasic nature of the ρ1 modulation (Bermack and Debonnel, 2005; Maurice et al., 1994a, b). Whatever the exact mechanism responsible for the bell-shaped nature of the SA 4503 dose–response curve, our results point toward an optimal dose close to 10 mg/kg, at which DRN 5-HT neuron frequency was almost doubled. This finding is in apparent contradiction with the results obtained by Skuza and Rogóz (2002) in the FST model. These authors found that the most efficient dose of SA 4503 for reducing immobility was 3 mg/kg, and that the administration of 10 mg/kg had no influence on this parameter. However, it is important to note that they chose to inject the animals three times with the drug, at 24, 5 and 1 h before the testing phase (Skuza and Rogóz, 2002). This protocol is relatively common when performing the FST, as it has been shown to improve the AD-like effect of classical AD (Cryan et al., 2002). Even if we do not dispose of accurate data on SA 4503 pharmacokinetic and half-life, one may suggest that three consecutive administrations of 3 mg/kg within a 24 h time-frame result in a ‘real’ dose closer to 9 mg/kg than to 3 mg/kg at the onset of testing. If so, our results on DRN 5-HT neuron firing rate would be actually in good agreement with those of Skuza and Rogóz (2002), the ‘real’ doses they used being around 9 mg/kg (optimal) and 30 mg/kg (no effect).

Another interesting feature of the excitatory action of ρ1 receptor agonists on DRN 5-HT neuron activity resides in the rapidity of its onset. Indeed, similarly to what had been previously observed with (+)-pentazocine, DTG or 4-IBP (Bermack and Debonnel, 2001), we found that SA 4503 was able to produce its effect after only 2 d of continuous administration. By comparison, the AD mirtazapine allows 5-HT neuron firing rate to remain unchanged in the same conditions, the minimal duration of chronic treatment required to obtain an increase (+60%) appearing to be 21 d (Besson et al., 2000). The fast onset of action of SA 4503 is even more striking when compared with the profile of selective serotonin reuptake inhibitors (SSRIs), currently and by far the most used AD drugs. These compounds are well known to induce an initial decrease, actually almost a suppression, of 5-HT electrical activity, due to an over-stimulation of somatodendritic 5-HT1A autoreceptors (Blier and de Montigny, 1999; El Mansari et al., 2005). A delay of 2–3 wk is necessary before 5-HT neurons recover their normal activity, when autoreceptors become fully desensitized (Artigas et al., 2002; Blier and de Montigny, 1999). This phenomenon has been extensively studied, as it is believed to underlie the delayed therapeutic action of classical AD in clinical use (Blier and de Montigny, 1999; El Mansari et al., 2005). The fact that SA 4503 enhanced DRN 5-HT neuron firing rate after only 2 d of treatment raises therefore the interesting hypothesis that it might constitute a potentially fast-acting AD. Such a possibility was further confirmed by evaluating the resulting influence of SA 4503 treatment on post-synaptic 5-HT transmission. Thus, after only 2 d treatment at the optimal dose of 10 mg/kg, SA 4503 induced the appearance of a 5-HT1A-mediated inhibitory post-synaptic tonus on pyramidal neuron activity within the CA3 subfield of the hippocampus. This parameter was increased by 70–80% after an acute injection of WAY 100635, comparable to the effect (+100%) observed in rats treated with the SSRI paroxetine (Haddjeri et al., 1998). The manifestation of such an inhibitory tonus appears to be typical and selective of AD therapy, and it can be observed with numerous treatments, like SSRIs, tricyclics, or electroconvulsive shocks (Besson et al., 2000; Haddjeri et al., 1998). However, and again, it is normally obtained only after 2–3 wk sustained administration (Besson et al., 2000; El Mansari et al., 2005; Haddjeri et al., 1998).

Hence, it appears that the rapid onset of action of SA 4503 on 5-HT neuron activity resulted in an enhanced efficacy of 5-HT transmission at the postsynaptic level within the same time-frame. In keeping with this finding, we also observed that SA 4503 augments cell proliferation in the SGZ of the adult hippocampus after 3 d chronic administration. Interestingly, even though we did not test all the doses used in the DRN 5-HT electrophysiological experiments, the mitogenetic property of SA 4503 seems to follow a similar dose–response curve with its facilitatory action on 5-HT neurons. The dose of 10 mg/kg was optimal when compared to that of 3 mg/kg, which by contrast was only marginally significant. The
fact that SA 4503 increased mitotic activity in the SGZ constitutes an additional and strong argument to propose an AD potential for this compound, as it is now well established that this is a typical property of AD molecules (Duman et al., 2001; Malberg et al., 2000; Nakagawa et al., 2002; Santarelli et al., 2003). To our knowledge, this is the first time that it has been described after a treatment with an \( \alpha_1 \) receptor agonist. In addition, the amplitude of the effect (+94% at 10 mg/kg) was substantially higher than the values reported after chronic treatment with classical AD (around +35–50%, Malberg et al., 2000; Santarelli et al., 2003). Moreover, and in line with the electrophysiological data discussed above, the enhanced cell proliferation was observable after only 3 d treatment. Classical AD, on the other hand, produce their neurogenetic action within 2–3 wk of continuous administration (Malberg et al., 2000; Nakagawa et al., 2002; Santarelli et al., 2003), an effect which may be a consequence of the augmented stimulation of post-synaptic 5-HT\(_{1A} \) receptors (Santarelli et al., 2003). Indeed, that SA 4503 increased central 5-HT neurotransmission and hippocampal cell proliferation in parallel (i.e. both within 2–3 d) further strengthens the hypothesis that 5-HT plays a central role in the expression of the latter (Santarelli et al., 2003). It should also be mentioned that \( \alpha_1 \) agonists are able to facilitate various cellular processes, underlying neuronal plasticity elicited by several growth factors in vitro (Takebayashi et al., 2002, 2004). Therefore, it cannot be excluded that \( \alpha_1 \) receptors exert a more global, positive influence on brain cell development through multiple mechanisms, including actual mitogenesis as well as cellular growth and differentiation processes.

Finally, SA 4503 was able to reduce time of immobility in the FST paradigm, through a more prominent swimming behaviour. Similar results have already been published in previous studies, which also showed that this effect is fully blocked by selective \( \alpha_1 \) receptor antagonism (Matsuno et al., 1996b), and is not due to a non-specific activation of locomotor function (Skuza and Rogóz, 2002). However, the dose–response curve reported by Skuza and Rogóz (2002) differed from our own, which in comparison seems to have been shifted to the right, as suggested by the data we obtained at 3 and 10 mg/kg (we did not perform an extensive dose–response study in the FST, contrary to what we did for DRN 5-HT electrophysiological experiments). This apparent discrepancy is probably related to the fact that we administered SA 4503 only once before testing, whereas these authors injected it three times before (see above). Interestingly, the ability of SA 4503 to reduce time of immobility after only one injection might constitute an additional argument to support the idea that its AD potential could have a rapid onset of action. The fact that classical AD such as SSRIs are effective in the FST is actually somewhat intriguing, when considering the 4–6 wk delay of action displayed by these molecules in clinical use (see above). Based on the observation that SSRIs are barely efficient after a single administration, the ‘three-times’ protocol has been developed for the FST, and has been proposed to reflect, although at a miniature time-scale, the need for a chronic treatment in depressed patients (Cryan et al., 2002). In this context, the effectiveness of SA 4503 at 30 min after a single injection could therefore indicate an increased rapidity of action. Obviously, this hypothesis has to be verified in truly ‘chronic’ behavioural models of depression, such as the chronic mild stress or olfactory bulbectomy. It is, however, consistent with the rapidity of the changes observed on 5-HT neurotransmission. Interestingly, SA 4503 had no influence on climbing behaviour, which is believed to be related to an increased noradrenergic tone, but seems to be independent from 5-HT modulation (Cryan et al., 2002). Since the picture has been reported to be inverted regarding swimming (Cryan et al., 2002), our observations further strengthen the idea that the 5-HT system plays a central role in the ‘antidepressant-like’ properties of SA 4503. It should be kept in mind, however, that this molecule is also able to modify the activity of other aminergic systems, notably the dopaminergic one (Minabe et al., 1999).

In summary, SA 4503 displays a number of characteristics typical of AD molecules in different electrophysiological, morphological and behavioural paradigms, in agreement with the general hypothesis that \( \alpha_1 \) receptor agonists constitute a new class of AD (Takebayashi et al., 2004). Additionally, a number of data suggest that its AD potential may have a rapid onset of action. This promising possibility needs to be further investigated in the near future by using appropriate behavioural models.

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Statement of Interest

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