Pharmacological Interaction With the Sigma1 (σ1)-Receptor in the Acute Behavioral Effects of Antidepressants

Vanessa Villard1,2,3, Johann Meunier1,2,3, Nathalie Chevallier1,2,3, and Tangui Maurice1,2,3,*

1Institut National de la Santé et de la Recherche Médicale, Unit 710, Montpellier 34095, France
2Université de Montpellier II, Montpellier 34095, France
3Ecole Pratique des Hautes Etudes, Montpellier 34095, France

Received July 19, 2010; Accepted August 16, 2010

Abstract. Selective agonists of the sigma-1 (σ1) ligand–operated chaperone protein, like igmesine or PRE-084, are antidepressants in preclinical depression models. σ1-Protein activation may contribute to the antidepressant efficacy of drugs known to act as selective serotonin-reuptake inhibitors (SSRI) or noradrenaline reuptake inhibitors through direct or indirect involvement of the σ1-receptor in the drug effect. We here compared antidepressant effects in two behavioral procedures, the forced swimming test (FST) and conditioned fear stress (CFS). The involvement of the σ1-receptor was examined using a co-treatment with the σ1-antagonist BD1047 or using σ1-knockout (KO) mice. Igmesine but not PRE-084 decreased FST immobility. The SSRI fluoxetine and sertraline, but not fluvoxamine, and the tricyclic antidepressants imipramine, desipramine, and amitriptyline were also effective. Only the effect of igmesine was blocked by BD1047 or in σ1-KO mice. Igmesine, PRE-084, fluvoxamine, and sertraline decreased the CFS immobility in a BD1047- and σ1-KO–sensitive manner. Among tricyclics, only amitriptyline was effective and its effect was unaffected by BD1047 or in σ1-KO mice. The behavioral effects induced by mixed σ1-receptor/SSRI antidepressants, like fluvoxamine or sertraline, may therefore involve a non-selective action at both targets. Moreover, the CFS appears to more reliably uncover a σ1 pharmacological component in antidepressant screening.

Keywords: σ1-receptor, forced swimming, conditioned fear stress, antidepressant, σ1-knockout mouse

Introduction

Present clinical treatments for major depression are based on drugs belonging to several pharmacological classes and presenting different therapeutic efficacies. Among them are selective serotonin-reuptake inhibitors (SSRI), like fluoxetine, fluvoxamine, sertraline, paroxetine, and citalopram, and compounds belonging to the tricyclic antidepressant class, acting as non-selective noradrenaline-serotonin-reuptake inhibitors, including imipramine, desipramine, and amitriptyline. Development of new compounds led to appreciable improvements in the side effect and toxicity profiles but to limited gains in efficacy as compared to previously marketed drugs (1 − 3). Moreover, the limited rate of responders and the long-lasting therapeutic delay encountered before any significant improvement over placebo remained the most obvious problems unresolved by current antidepressant therapies. Other pharmacological strategies are thus presently investigated, including calcium-channel modulators, N-methyl-D-aspartate (NMDA)-receptor antagonists, peptide receptor modulators like neurokinin-receptor antagonists, or hormone receptor modulators like corticotropin-releasing hormone–receptor antagonists (1, 3, 4). Several studies outlined the promising preclinical profiles of compounds acting at the sigma1 (σ1)-receptor (5 − 14).

The σ1-receptor is an intracellular chaperone protein originating from endoplasmic reticulum membranes, but also localized on nuclear, mitochondrial, and plasma membranes (15 − 17). In physiological conditions, it forms a heterotrimeric macromolecular complex with the
inotrope, fluvoxamine, sertraline, imipramine, or fluoxetine, on BDNF signaling via the PLC-γ/IP3/Ca2+ is mediated via the σ1-receptor, and that the σ1-receptor plays an important role in BDNF signaling leading to glutamate release and sustaining its involvement in antidepressant action. Second, Hashimoto et al. (35) reported that fluvoxamine, but not paroxetine, attenuated the cognitive deficits in mice induced after a chronic treatment with phencyclidine, in a similar manner as observed with the σ1-receptor agonists SA4503 and dehydroepiandrosterone sulfate and the effect was blocked by the selective σ1-receptor antagonist N,N-dipropyl-2-[4-methoxy-3-(2-phenylethoxy)-phenyl]-ethylamine (NE-100).

The purpose of the present study was to determine whether the activity at the σ1-receptor is involved in the acute effects of antidepressants, whatever their primary target, that is, serotonin or noradrenaline transporter, could be. SSRI and tricyclic antidepressants were therefore tested in two behavioral tests, the FST, measuring acute behavioral despair as a model of antidepressant-like activity, and the conditioned fear stress (CFS) response (36), measuring psychological stress–induced motor suppression, a model of the anxiogenic response. The effects of antidepressants were compared to the behavioral efficacy of selective σ1-agonists, igmesine and 2-(4-morpholinoethyl)-1-phenylcyclohexane-1-carboxylate hydrochloride (PRE-084). When drugs showed a significant effect, the effect of a pretreatment with the σ1-antagonist N-[2-(3,4-dichlorophenyl)ethyl]-N-methyl-2-(dimethylamino)ethylamine (BD1047) was examined and the drugs were tested in σ1-knockout (KO) mice.

Materials and Methods

Animals

Male Swiss mice (Breeding centre of the Faculty of Pharmacy, Montpellier) were used at 5 – 7 weeks of age. Heterozygous (+/−) σ1-KO mice, OprGtIRISHetageo33Lex, were purchased from the Texas Institute for Genomic Medicine (Houston, TX, USA). Initially generated by a gene trapping strategy, the animals were maintained in a mixed C57BL/6j × 129s/Sv background. Genotyping was performed by PCR after modification of the manufacturer’s instructions, as described (N. Chevallier et al., submitted article). Littermates +/+ (wild-type, WT) and −/− animals, resulting from heterozygous mating, were used at 2 months of age in this study. Animals were housed in groups in plastic cages with free access to food and water and kept in a regulated environment (23 ± 1°C, 40% – 60% humidity, 12-h light/dark cycle with lights on at 7:00 AM). Experiments were carried out between 10:00 AM and 5:00 PM, in a sound-attenuated and air-regulated experimental room, to which mice were habiti-
ated for at least 30 min. All animal procedures were conducted in strict adherence with the European Council Directive of 24 November 1986 (86-609/EEC).

**Drugs**

(+) N-cyclopropylmethyl-N-methyl-1,4-diphenyl-1-ethyl-but-3-en-1-ylamine hydrochloride (igmesine) was provided by Dr. F.J. Roman (Pfizer, Fresnes, France). 2-(4-Morpholinoethyl)-1-phenyleclohexane-1-carboxylate hydrochloride (PRE-084) was provided by Dr. Tsung-Ping Su (NID/NIH, Baltimore, MD, USA). N-[2-(3,4-Dichlorophenyl)ethyl]-N-methyl-2-(dimethylamino)ethylamine (BD1047) was provided by Dr. Wayne D. Bowen (NIDDK/NIH, Bethesda, MA, USA). Other drugs were from commercially available sources: imipramine and desipramine (Sigma/Aldrich, St-Quentin Fallavier, France); amitriptyline (Laroxyl®; Roche, Neuilly-sur-Seine, France), fluoxetine (Prozac®, Eli Lilly, Basingstoke, UK), fluvoxamine (Floxyfral®, Solvay Pharma, Suresnes, France); haloperidol (Haldol®, Janssen, Boulogne-Billancourt, France); sertraline (Zoloft®, Pfizer, Paris, France). Powder from capsules (sertraline) or tablets (imipramine, fluoxetine) was solubilized using an ultrasonic homogenizer and the carboxymethylcellulose excipient was sedimented. Vehicle solutions were distilled water (for igmesine, amitriptyline); 0.9% saline solution (for PRE-084, BD1047, and desipramine); or 12 mg/ml lactose, 5.6 mg/ml cornstarch, and 0.2 mg/ml magnesium stearate in distilled water (for imipramine, sertraline, fluoxetine, and fluvoxamine). Drugs were injected intraperitoneally (i.p.) in a volume of 100 μl per 20 g of body weight. Doses refer to the free base form and were calculated from the known amount of compound per capsule or tablets.

**FST**

The procedure previously used to assess behavioral despair in mice was used (9, 32). On day 1, each mouse was placed individually in a glass cylinder (diameter of 12 cm, height of 24 cm) filled with water at a height of 12 cm. Water temperature was maintained at 23 ± 1°C. Animals were forced to swim for 15 min and then returned to their home cage. On day 2, animals were placed again into the water and forced to swim for 6 min. The mouse was considered as immobile when it stopped struggling and moved only to remain floating in the water, keeping its head above water. The session was videotrapped (ViewPoint, Champagne-au-Mont-d’Or, France) and the quantity of movement quantified min per min by the software. The duration of immobility was analyzed during the last 5 min of the session. The mobility of mice was checked 5 min after returning them to their home cage. None of the animals included in the study exhibited a particular hypomobility response due to hypothermia. Direct measure of hypothermia was however not performed. Drugs were administered 30 min before the session on the second day, BD1047 being administered simultaneously with each other drug.

**CFS**

The apparatus was a transparent acrylic rectangular cage (30 × 30 × 40 high cm) equipped with a metal wire floor. On day 1, each mouse was placed into the test cage and received intermittent electric shocks (0.1 Hz, 200 ms, 60 V DC) for 10 min through an isolated pulse stimulator (Model 2100; AM-Systems, Everett, WA, USA). When the mouse was placed in the test cage, the current resistance varied from 100 to 250 kΩ. Therefore each mouse received electric shocks varying from 0.24 to 0.60 mA. The shock sensitivity was assessed by summing the numbers of vocalizations and flinching reactions of the animal. None of the treatments used in the present study affected the shock sensitivity of the animals (data not shown). The test session was performed 24 h after the first session. Animals were placed again into the test cage, but no foot shock was delivered. The motor activity of mice was measured during 6 min using an infrared beams activity device (Opto-varimex; Columbus Instruments, Columbus, OH, USA), in which the cage was inserted. The non-shocked control groups were operated similarly, except for the absence of shock treatment. Drugs were administered 30 min before the test session, BD1047 being administered simultaneously with each other drug.

**Statistical analyses**

Results are expressed as the mean ± S.E.M. Dose–response experiments in the FST were analyzed using a one-way analysis of variance (ANOVA, F-value) followed by a Dunnett’s post-hoc test for group comparisons. Antagonism experiments were analyzed using a two-way ANOVA, with the dose of agonist and dose of antagonist as independent factors, followed by a Newman-Keuls’ post-hoc test. Dose–response experiments in the CFS test were analyzed using a two-way ANOVA, with shock and dose of compound as independent factors. Antagonism experiments were analyzed using a three-way ANOVA, with the shock, agonist, and antagonist as independent factors. Group comparisons were performed using the Newman-Keuls’ post-hoc test, when justified by the ANOVA. The F-values were detailed in the legend of each figure, for clarity. F-values lower than 1 could not lead to significant difference whatever the degrees of freedom and were indicated as such (F < 1).
Results

Effects of \(\sigma_1\)-compounds in the FST

BD1047, in the 1 – 10 mg/kg dose, failed to affect the immobility duration (Fig. 1a). Igmesine diminished in a dose-dependent manner the immobility duration (Fig. 1b). A 53% decrease as compared to the control was measured at the highest dose tested. PRE-084 in the 10 – 40 mg/kg dose-range failed to affect the immobility duration (Fig. 1c). The igmesine effect was blocked by the co-administration of a low dose, 4 mg/kg, of BD1047 (Fig. 1d). The \(\sigma_1\)-KO mice showed a significantly lower immobility response in the FST (grey column, Fig. 1e). In these animals, igmesine failed to decrease immobility, contrarily to WT animals (Fig. 1e).

Effects of antidepressants in the FST

Fluoxetine reduced in a dose-dependent manner the immobility duration (Fig. 2a). A 28% reduction in immobility as compared to the control was observed at the highest dose tested. Fluvoxamine was ineffective in the dose range tested (Fig. 2b). Sertraline dose-dependently reduced the immobility duration (Fig. 2c), with a 39% effect at 40 mg/kg. The fluoxetine effect was not affected by the administration of BD1047 (Fig. 2d) and appeared even significantly augmented in \(\sigma_1\)-KO mice as compared with WT controls (Fig. 2e). Similarly, the effect of sertraline was not affected by the co-administration of BD1047 (Fig. 2f), but it was highly significantly augmented in \(\sigma_1\)-KO mice as compared with WT controls (Fig. 2g).

Imipramine reduced the immobility duration (Fig. 3a), with a 56% reduction at 40 mg/kg. Desipramine also showed a significant effect (Fig. 3b), with a 37% reduction at 40 mg/kg. Amitriptyline in the 5 – 20 mg/kg dose-range reduced immobility duration (Fig. 3c). The co-administration of BD1047 failed to affect the decrease in immobility induced by imipramine (Fig. 3d), desipramine (Fig. 3e), or amitriptyline (Fig. 3f). When the drugs were tested in KO mice, no difference in the activity was measured between WT and \(\sigma_1\)-KO mice for imipramine (Fig. 3g), desipramine (Fig. 3h), or amitriptyline (Fig. 3i).

Effects of \(\sigma_1\)-compounds in the CFS

BD1047, at 4 or 10 mg/kg i.p., failed to affect the mobility of control, non-shocked animals or the motor suppression observed in animals that experienced the foot shock on day one (Fig. 4a). On the contrary, igmesine significantly attenuated the motor suppression at the dose of 40 mg/kg, without any effect in control, non-shocked animals (Fig. 4b). A 59% reduction of the motor suppression was measured as compared to controls at the highest

\[ F < 1 \] in (a); \[ F(1,36) = 15.7, P < 0.0001 \] in (b); \[ F < 1 \] in (c); \[ F(1,36) = 12.1, P < 0.01 \] for igmesine, \[ F(1,36) = 8.28, P < 0.01 \] for BD1047 and \[ F(1,36) = 8.21, P < 0.01 \] for the interaction in (d); \[ F(1,66) = 10.5, P < 0.01 \] for igmesine, \[ F < 1 \] for the phenotype and \[ F(1,66) = 7.15, P < 0.01 \] for the interaction in (e). *\( P < 0.05 \), **\( P < 0.01 \) vs. the V-treated group; ***\( P < 0.01 \) vs. the igmesine (40 mg/kg)–treated group; Dunnett’s test in (b), Newman-Keuls’ test in (d and e).

![Fig. 1. Effect of the \(\sigma_1\)-receptor ligand BD1047 (a), igmesine (b), or PRE-084 (c) on the immobility time in mice submitted to the FST; effect of igmesine in co-administration with BD1047 (d) and in \(\sigma_1\)-KO mice (e). Mice were submitted to a 15-min duration FST session and, one day after, to a 6-min duration FST session. Drugs (mg/kg), or their respective vehicle solution (V), were administered i.p. 30 min before the 6-min test and the duration of immobility was recorded during the last 5 min. The number of animals per group is indicated within each column. Statistical analyses: F < 1 in (a); F(1,36) = 15.7, P < 0.0001 in (b); F < 1 in (c); F(1,36) = 12.1, P < 0.01 for igmesine, F(1,36) = 8.28, P < 0.01 for BD1047 and F(1,36) = 8.21, P < 0.01 for the interaction in (d); F(1,66) = 10.5, P < 0.01 for igmesine, F < 1 for the phenotype and F(1,66) = 7.15, P < 0.01 for the interaction in (e). *\( P < 0.05 \), **\( P < 0.01 \) vs. the V-treated group; ***\( P < 0.01 \) vs. the igmesine (40 mg/kg)–treated group; Dunnett’s test in (b), Newman-Keuls’ test in (d and e).]
dose tested. PRE-084 in the 10 – 40 mg/kg dose-range also attenuated significantly the motor suppression observed in shocked mice, without affecting the behavior of control, non-shocked animals (Fig. 4c). The effect was bell-shaped and a 73% reversion of the motor suppression was measured at 20 mg/kg. The igmesine effect, at 40 mg/kg, was blocked by the co-administration with a low dose of BD1047 (Fig. 4d). Similarly, the PRE-084 effect at 20 mg/kg was blocked in presence of BD1047 (Fig. 4e). The σ₁-KO mice showed a significant motor suppression in the CFS (white column, Fig. 4f). In these animals, neither igmesine nor PRE-084 could attenuate the freezing behavior, contrarily to WT animals (Fig. 4f).
Effects of antidepressants in the CFS

Fluoxetine at 20 and 40 mg/kg, i.p. failed to affect the mobility of non-shocked animals or the motor suppression observed in shocked animals (Fig. 5a). Fluvoxamine significantly attenuated the motor suppression at the dose of 40 mg/kg, without any effect in control, non-shocked
285Antidepressants Interaction With the σ\(_1\) Receptor

animals (Fig. 5b). A 53% reduction of the motor suppression was measured as compared to controls at the highest dose tested. Sertraline also dose-dependently reduced the motor suppression (Fig. 5c), with a 37% effect at 40 mg/kg. The fluvoxamine effect at 40 mg/kg could be blocked by BD1047 (Fig. 5d). The BD1047 co-treatment also blocked the sertraline effect (Fig. 5e). In \(\sigma_1\)-KO mice (Fig. 5f), the effect of fluvoxamine was blocked, while sertraline showed a non-significant reduction of the motor suppression effect.

Imipramine at 20 and 40 mg/kg, i.p. failed to affect the mobility of non-shocked animals or the motor suppression observed in shocked animals (Fig. 6a). Desipramine, interestingly, tended to augment the motor suppression, but the effect did not reach statistical significance (Fig. 6b). Amitriptyline significantly attenuated the motor suppression observed in shocked mice at 10 mg/kg, without affecting the behavior of control, non-shocked animals (Fig. 6c). The effect was bell-shaped, leading to a 45% reversion as compared to controls. The BD1047

Fig. 4. Effect of the \(\sigma_1\)-receptor ligand BD1047 (a), igmesine (b), or PRE-084 (c) on the CFS-induced motor suppression in mice; effect of igmesine or PRE-084 in co-administration with BD1047 (d and e) and in \(\sigma_1\)-KO mice (f). Mice were submitted to a 10-min duration scrambled foot shock session and, one day after, to a 6-min duration test session. Drugs (mg/kg), or their respective vehicle solution (V), were administered i.p. 30 min before the 6-min test and the motor suppression was monitored by infrared beam counts. The number of animals per group is indicated within each column. Control animals did not receive shocks on day one (non-shocked). Non-shocked animals were not analyzed in (f). Statistical analyses: \(F_{(1,40)} = 98.1, P < 0.0001\) for the shock, \(F < 1\) for the dose and shock \(\times\) dose interaction in (a); \(F_{(1,40)} = 65.1, P < 0.0001\) for the shock, \(F_{(2,42)} = 4.27, P < 0.05\) for the dose, \(F_{(2,42)} = 1.75, P > 0.05\) for the shock \(\times\) dose interaction in (b); \(F_{(1,60)} = 47.1, P < 0.0001\) for the shock, \(F_{(1,60)} = 2.79, P < 0.05\) for the dose, \(F_{(1,60)} = 1.04, P > 0.05\) for the shock \(\times\) dose interaction in (c); \(F_{(1,76)} = 115, P < 0.0001\) for the shock, \(F_{(2,76)} = 5.88, P < 0.05\) for igmesine, \(F_{(2,76)} = 2.05, P > 0.05\) for BD1047, \(F_{(2,76)} = 2.03, P > 0.05\) for the shock \(\times\) igmesine interaction, \(F_{(2,76)} = 5.69, P < 0.05\) for the shock \(\times\) BD1047 interaction, \(F_{(2,76)} = 4.38, P > 0.05\) for the igmesine \(\times\) BD1047 interaction, \(F_{(2,76)} = 4.62, P < 0.05\) for the shock \(\times\) igmesine \(\times\) BD1047 interaction in (d); \(F_{(1,56)} = 167, P < 0.0001\) for the shock, \(F_{(1,56)} = 5.30, P < 0.05\) for PRE-084, \(F_{(1,56)} = 3.76, P > 0.05\) for BD1047, \(F_{(1,56)} = 7.41, P < 0.01\) for the shock \(\times\) igmesine interaction, \(F_{(1,56)} = 1.75, P > 0.05\) for the shock \(\times\) BD1047 interaction, \(F_{(1,56)} = 9.07, P < 0.01\) for the PRE-084 \(\times\) BD1047 interaction, \(F_{(1,56)} = 1.96, P > 0.05\) for the shock \(\times\) PRE-084 \(\times\) BD1047 interaction in (e); \(F_{(2,54)} = 11.4, P < 0.0001\) for the drug treatments, \(F_{(1,54)} = 87.3, P < 0.0001\) for the phenotype, \(F_{(2,54)} = 9.20, P < 0.0001\) for the interaction in (f). *\(P < 0.05\), **\(P < 0.01\) vs. the respective non-shocked group; P \(< 0.05\), **\(P < 0.01\) vs. the respective V-treated group; **\(P < 0.01\) vs. the shocked igmesine (40 mg/kg)– or PRE-084 (20 mg/kg)–treated group; \(\#P < 0.05, \#\#P < 0.01\) vs. the V-treated WT group; Newman-Keuls’ test.
co-treatment failed to affect the amitriptyline-induced attenuation of motor suppression (Fig. 6d). When amitriptyline was tested in KO mice (Fig. 6e), the drug showed a highly significant attenuation of the motor suppression, comparable to the effect observed in WT animals.

**Discussion**

The mechanism and therapeutical relevance of the $\sigma_1$-receptor involvement in the antidepressant response is currently under extensive investigation. Selective $\sigma_1$-receptor agonists show marked antidepressant activity. However, it is at present unclear how activation of the $\sigma_1$-receptor directly promotes the antidepressant effect. Moreover, a pharmacological interaction with the $\sigma_1$-receptor may facilitate the antidepressant action of drugs acting primarily as SSRI, non-selective noradrenaline- and serotonin-reuptake inhibitor, monoamine oxidase inhibitor, or atypical antidepressants. Indeed, numerous antidepressants, whatever their chemical class, show moderate to high affinity for $\sigma_1$-receptors and an interac-
Antidepressants Interaction With the $\sigma_1$ Receptor

The interaction with this target may participate to their pharmacological efficacy as antidepressant. We confirmed in preliminary experiments the binding affinities described in several previous reports (8, 10, 27, 28). Using $[^{3}H](\pm)$-pentazocine as a selective $\sigma_1$-receptor radioligand, we measured high affinities for sertraline and fluvoxamine, with $K_i$ values lower than 20 nM and moderate affinities for fluoxetine, amitriptyline, and imipramine, with $K_i$ values in the 100 – 200 nM range (data not shown). Moreover, after chronic treatment, several antidepressants were shown to regulate the $\sigma_1$-receptor expression. Several SSRI like fluoxetine or sertraline and tricyclic antidepressants like imipramine and more surprisingly desipramine decreased the expression of $\sigma_1$-receptors in several regions of the limbic system (8, 29). The involvement of an interaction with the $\sigma_1$-receptor in the pharmacological profile of the drugs must however be considered relatively to interaction with the other targets and thus be demonstrated through objective behavioral responses.

In the present study, we examined the efficacy of antidepressants in two acute stress tests in order to determine whether a pharmacological interaction with the $\sigma_1$-receptor could directly be evidenced in their pharmacological activity. The FST is the most frequently used screening model for assessment of antidepressant activity. All classes of antidepressant drugs, including tricyclics, SSRI, monoamine oxidase inhibitors, and atypical antidepressants, were found to be effective in attenuating the immobility induced by FST (14, 37 – 43), although with different responsiveness among drugs (44).
CFS-induced motor suppression, an index of the anxiogenic response induced by psychological stress, could also be attenuated by treatments with antidepressants acting as an SSRI, like citalopram or fluvoxamine (45 – 48), suggesting that the freezing behavior is mediated by serotonin-receptor inactivation (46, 47). The main difference between FST and CFS remains however that the stressor is still present during the FST session, that is, animals are floating in water, but not during the CFS session, that is, no shock is applied on day two.

On the one hand, selective \( \sigma_1 \)-receptor agonists, like (+)-pentazocine, (+)-SKF-10,047, SA4503, or igmesine, reduced the immobility time in the FST (9, 14, 24). We confirmed the efficacy of igmesine in the present study. The other reference \( \sigma_1 \)-agonist tested, PRE-084, failed to decrease the duration of immobility. As previously reported (14), the steroidal tonus markedly interferes with the \( \sigma_1 \)-response in this procedure. Among steroids, progesterone is a very potent antagonist and progesterone levels are markedly increased during the FST stress (14). It appeared indeed that the intrinsic efficacy of PRE-084 is lower than that of igmesine for the FST response, and PRE-084 showed a significant antidepressant-like activity only in adrenalectomized/castrated Swiss OF1 animals, when the steroidal tonus is markedly reduced (14). Moreover, the drug showed a significant antidepressant-like efficacy in C57BL/6 mice, also known as presenting a lower steroidal tonus (49). The non-selective 5-HT\(_{1A}/\sigma_1\)-receptor agonist OPC-14523 also showed an antidepressant-like effect in the FST, through its \( \sigma_1 \)-receptor component (13). The \( \sigma_1 \)-receptor antagonist NE-100 or BD1047 reversed the drug-induced reduction of immobility. Igmesine, which is very efficient in the FST and tail suspension tests in rats and mice (14, 50), has been tested in a clinical trial in humans with promising results (51). On the other hand, \( \sigma_1 \)-receptors also play an important role in the CFS response (for reviews, see 6, 24). Several \( \sigma_1 \)-receptor agonists such as (+)-SKF-10,047, dextromethorphan, or igmesine attenuated the motor suppression in rodents, the effects being antagonized by the \( \sigma_1 \)-receptor antagonists NE-100 or BD1047 (5, 33) or in \( \sigma_1 \)-KO mice (this study).

Here, two distinct points were addressed. First, the involvement of the \( \sigma_1 \)-receptor in the acute effects of antidepressants was questioned using a pharmacological approach, the determination of the ability of BD1047 to antagonize the observed effects, and a genetic approach, by measuring the drug effect in \( \sigma_1 \)-KO mice, and in both the FST and CFS tests. Second, the activity profiles in the two tests were compared in order to determine whether the \( \sigma_1 \) pharmacological component was similarly involved. The results of the study summarized in Table 1 clearly showed that the efficacy of antidepressants to attenuate the immobility in the FST is not primarily related to their \( \sigma_1 \)-receptor pharmacological interaction. Among the \( \sigma_1 \)-receptor agonists tested, only igmesine showed beneficial effect in FST. Fluoxetine, sertraline, and the three tricyclic antidepressant drugs tested reduced the immobility duration in a BD1047- and \( \sigma_1 \)-KO-insensitive manner, that is, their activity involves their other pharmacological targets, the serotonin transporter or the serotonin and noradrenalin transporters and therefore through an increased efficacy of serotoninergic neurotransmission or of both serotoninergic and noradrenalinergic transmission.

<table>
<thead>
<tr>
<th>Drug</th>
<th>FST activity</th>
<th>Drug effect blocked by BD1047</th>
<th>CFS activity</th>
<th>Drug effect blocked by BD1047</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \sigma_1 )-Ligands</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Igmesine</td>
<td>+</td>
<td>yes</td>
<td>+</td>
<td>yes</td>
</tr>
<tr>
<td>PRE-084</td>
<td>–</td>
<td>yes</td>
<td>+</td>
<td>yes</td>
</tr>
<tr>
<td>SSRI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>+</td>
<td>no</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Fluvoxamine</td>
<td>–</td>
<td>+</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Sertraline</td>
<td>+</td>
<td>no</td>
<td>+</td>
<td>yes</td>
</tr>
<tr>
<td>Tricyclic antidepressants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imipramine</td>
<td>+</td>
<td>no</td>
<td>no</td>
<td>–</td>
</tr>
<tr>
<td>Desipramine</td>
<td>+</td>
<td>no</td>
<td>no</td>
<td>–</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>+</td>
<td>no</td>
<td>no</td>
<td>+</td>
</tr>
</tbody>
</table>

+: effect, –: no effect.
Adrenergic systems. The explanation of the lack of activity of these drugs at the \( \sigma_1 \)-receptor in these conditions is still to be determined. The important acute stress induced during the test session provokes releases of neurohormones and hypothalamic–pituitary–adrenal axis activation that may directly compete with the activity at the \( \sigma_1 \)-receptor. Indeed, selective drugs showed a moderate activity in the FST, as compared with what could be expected from their in vitro affinities (high active dose for igmesine, no activity for PRE-084 in this study); and unlike fluoxetine and sertraline, non-selective drugs failed to show a significant interaction. Direct measures of \( \sigma_1 \)-receptor occupancy during acute stress could tentatively help to address this point.

It should be noted that the lack of efficacy of fluvoxamine in the FST has been previously reported to be highly dependent on the mouse strain (52). The drug appeared less effective in mouse strains presenting low \( B_{\text{max}} \) for \(^{[3]}\)Hparoxetine binding, that is, low density of serotonin transporters, such as ICR, ddY, and C57BL/6 (52). Whether this observation also applied to Swiss OF1 mice must be examined.

The CFS results showed, on the contrary, a marked involvement of the \( \sigma_1 \)-receptor. PRE-084, tested in the same dose-range as used in FST, was effective and its effect blocked by BD1047 and in \( \sigma_1 \)-KO mice. The SSRI sertraline and fluvoxamine that show a high affinity for the \( \sigma_1 \)-receptor were effective. Their effects were blocked by BD1047 and in \( \sigma_1 \)-KO mice, although sertraline was still able to induce a non-significant attenuation of the motor suppression. Interestingly, among the tricyclic antidepressants tested in the CFS, in the same dose-range as used for the FST, amitriptyline was the only effective one. The compound attenuated the motor suppression in a BD1047-and \( \sigma_1 \)-KO–insensitive manner, thus through an effect unrelated to the \( \sigma_1 \)-receptor. It could thus be concluded that an interaction with the \( \sigma_1 \)-receptor is more clearly demonstrated in the CFS paradigm than in the FST, at least considering SSRI compounds. Using this procedure, an interaction with the \( \sigma_1 \)-receptor could be measured in the behavioral effect of fluvoxamine and sertraline, the two compounds behaving as \( \sigma_1 \)-receptor agonists.

Both fluvoxamine and sertraline have been suggested to mediate their antidepressant activity through a pharmacological interaction with the \( \sigma_1 \)-receptor. Fluvoxamine binds to \( \sigma_1 \)-receptors in the human brain at therapeutic doses, as shown by a positron emission tomography study (53). Using the most characterized pharmacological test assessing \( \sigma_1 \)-receptor activity, the potentiation of NMDA-induced firing activity of CA3 pyramidal neurons in the rat hippocampus, Bergeron et al. (31) described that low doses of sertraline and clorgyline potentiated with a bell-shaped dose–response curve the NMDA effect. This potentiation was reversed by the \( \sigma_1 \)-receptor antagonists haloperidol and BMY-14802. Recently, Rogoz and Skuza (54) examined the effect of combined treatment of male Wistar rats with pramipexole and fluvoxamine or fluoxetine or sertraline in the FST. The co-treatments pramipexole plus fluoxetine or pramipexole plus sertraline exhibited antidepressant-like activity. The dopamine D\_2/D\_3–receptor antagonist sulpiride and 5-HT\_1A-receptor antagonist WAY 100635 inhibited the antidepressant-like effect induced by co-administration of pramipexole plus fluoxetine or pramipexole plus sertraline. The \( \sigma_1 \)-receptor antagonist progesterone or BD1047 counteracted the antidepressant-like effect induced by co-administration of pramipexole and sertraline but not pramipexole and fluoxetine. Therefore, the more pronounced antidepressant activity induced by the co-treatments pramipexole plus fluoxetine or pramipexole plus sertraline, than does treatment with pramipexole alone, involved complex dopamine D\_2/D\_3– and 5-HT\_1A-receptor antidepressant-like activities. Moreover, \( \sigma_1 \)-receptor constitutes one of the mechanisms by which co-administration of pramipexole plus sertraline induces antidepressant-like activity in the FST (54). These profiles could be explained by a \( \sigma_1 \)-agonist activity of DTG and sertraline and \( \sigma_1 \)-antagonist action of opipramol. Our present data in the CFS show a clear \( \sigma_1 \)-agonist activity of sertraline on a behavioral response known to be directly induced by activation of the \( \sigma_1 \)-protein. We observed a complete blockade of the sertraline effect (i) by a selective \( \sigma_1 \)-agonist, BD1047, and (ii) in \( \sigma_1 \)-KO mice. These observations are coherent with the reports of Bergeron et al. (31) and Rogoz and Skuza (54). Interestingly, several recent reports (35, 55, 56) demonstrated, on neurite sprouting in PC12 cells and from human clinical studies, that fluvoxamine’s action involve a \( \sigma_1 \)-agonist effect in its pharmacological activity, while sertraline was devoid of effect or showed opposite effects. The effects observed with sertraline could in fact appear coherent with an “inverse agonist” activity at \( \sigma_1 \)-sites. Indeed, sertraline at 10 \( \mu \)M significantly decreased the neurite outgrowth in PC12 cells (55). This could be due to an inverse agonistic action if this decrease could be blocked by a \( \sigma_1 \)-antagonist. This point was however not investigated by the authors. The \( \sigma_1 \) pharmacology is known to present such discrepancy in the drug activities, depending on the physiological response observed (for reviews addressing this point, see refs. 24 and 26). A typical example is the observation for the neurosteroid pregnenolone sulfate of an agonist effect on bradykinin-induced intracellular Ca\(^{2+}\) mobilization from the endoplasmic reticulum in NG-108 cells (16) or on the scopolamine or amyloid-\( \beta_{25,35} \)-induced amnesia in mice (24) and of an “inverse agonist” profile
on the potentiation of the NMDA-induced electrophysiological activity of CA3 pyramidal neurons in the rat hippocampus (57). The steroid indeed decreased the NMDA-induced response, contrarily to (+)-pentazocine, and this effect was blocked by haloperidol. The complex cellular role of the σ1-protein that acts as an intracellular Ca\(^{2+}\) sensor/modulator and molecular chaperone and at different localization within cells, mainly at the ER/mitochondria membrane focal contact points and at the plasma membrane (17), suggests a multiplicity of modes of activation and therefore of consequences of its activation by exogenous ligands, at the cellular level. Taking into account the particular consequences on neuronal networks, particularly monoaminergic pathways, and the drug bioavailability and pharmacological selectivity profile, it is not surprising that it could behave as an agonist on one response and behave as an inverse agonist on another. Finally, in the human study (56), a difference of efficacy between fluvoxamine and sertraline on the appearance of delusions in a patient with psychotic depression was reported. Fluvoxamine abolished delusions, while sertraline was devoid of effect on this symptom. The authors attributed the efficacy of fluvoxamine on delusions to its σ1-agonist activity (56). Since the clinical dose of fluvoxamine was two times higher than the sertraline dose, whereas the fluvoxamine affinity for σ1-site is also two times higher, it may therefore be suggested that sertraline did not interact with the σ1-sites at its clinically active dose, contrarily to fluvoxamine, explaining its lack of effect on delusions.

Moreover, Yagasaki et al. (34) examined the effects of pretreatment with antidepressants on the BDNF signaling through the PLC-γ/IP3/Ca\(^{2+}\) pathway. They reported that the BDNF-stimulated PLC-γ activation and the ensued increase in intracellular Ca\(^{2+}\) were potentiated by pretreatment with imipramine or fluvoxamine, so was the BDNF-induced glutamate release. BD1047 blocked the imipramine-dependent potentiation on the BDNF-induced PLC-γ activation and glutamate release. Over-expression of the σ1-receptor per se, without antidepressant pretreatment, enhanced BDNF-induced PLC-γ activation and glutamate release. These observations suggested that antidepressant pretreatment selectively but putatively indirectly enhanced the BDNF signaling on the PLC-γ/IP3/Ca\(^{2+}\) pathway via the σ1-receptor (34). Finally, Takebayashi et al. (58) reported that σ1-receptor might participate in the neurite sprouting and that antidepressants with σ1-receptor affinity may promote the neuronal sprouting via the σ1-receptor. The σ1-receptor agonist (+)-pentazocine or imipramine or fluvoxamine also dose- and time-dependently increased σ1-receptor expression in PC12 cells. These results suggested that NGF induces neurite sprouting by increasing σ1-receptors, the over-expression of σ1-receptor per se enhancing the NGF-induced neurite sprouting (58). It could therefore be concluded that activation of the σ1-receptor is an important step in the antidepressant action, whatever their primary pharmacological target could be. The use of CFS screening may allow a rapid identification of antidepressants directly interacting with σ1-receptors.

In conclusion, this study examined the effects induced by several antidepressants (σ1-receptor agonists, SSRI, and tricyclic antidepressants) in rodents submitted to two behavioral tests. The results confirmed that σ1-receptor agonists may present a significant antidepressant potential. Moreover, although they act primarily through inhibition of the serotonin transporter, SSRI, like fluvoxamine or sertraline, also involve a pharmacological interaction with the σ1-receptor. The CFS test seems to be highly reliable to uncover a σ1-pharmacological component in antidepressant screening, although it shows a poorly predictive validity of anxiolytic vs. antidepressant activity.

Acknowledgments

The authors acknowledge the technical assistance of Annick Creac’h, Fanny Malhaire-Fereux, and Lucie Rubio. Thanks are due to Drs. François J. Roman, Tsung-Ping Su, and Wayne D. Bowen for their gift of drugs used in this study. This work was supported by institutional funding from the Centre National de la Recherche Scientifique (CNRS, Paris, France) and Institut National de la Santé et de la Recherche Médicale (INSERM, Paris, France).

References

7 Kamei H, Noda Y, Kameyama T, Nabeshima T. Role of (+)-SKF-10,047-sensitive sub-population of σ1 receptors in amelioration
Antidepressants Interaction With the σ₁ Receptor

291


Skuzu G, Rogoz Z. Sigma receptor antagonists attenuate antidepressant-like effect induced by co-administration of 1,3-di-o-tolyguanidine (DTG) and memantine in the forced swimming test in rats. Pol J Pharmacol. 2003;55:1149–1152.


Urani A, Roman FJ, Phan VL, Su TP, Maurice T. The antidepressant-like effect induced by sigma₁ (σ₁) receptor agonists and neuroactive steroids in mice submitted to the forced swimming test. J Pharmacol Exp Ther. 2001;298:1269–1279.


Hayashi T, Su TP. Sigma-1 receptor chaperones at the ER-mitochondrion interface regulate Ca²⁺ signaling and cell survival. Cell. 2007;131:596–610.

Hayashi T, Su TP. Regulating ankyrin dynamics: roles of sigma₁ receptors. Proc Natl Acad Sci U S A. 2001;98:491–496.

Su TP, Hayashi T. Understanding the molecular mechanism of σ₁ receptors: towards a hypothesis that σ₁ receptors are intracellular amplifiers for signal transduction. Curr Med Chem. 2003;10:2075–2082.


Urani A, Romieu P, Roman FJ, Maurice T. Enhanced antidepressant effect of sigma₁ agonists in 3β,3α,4β-α-methyl-3α,4α,5α,6β-tetrahydro-1α-naphthylamine-reversible reduction of σ binding sites by chronic imipramine treatment in rat brain. Behav Brain Res. 2002;134:239–247.


Urani A, Romieu P, Roman FJ, Maurice T. Enhanced antidepressant effect of sigma₁ agonists in 3β,3α,4β-α-methyl-3α,4α,5α,6β-tetrahydro-1α-naphthylamine-reversible reduction of σ binding sites by chronic imipramine treatment in rat brain. Behav Brain Res. 2002;134:239–247.


Koe BK, Weissman A, Welch WM, Browne RG. Sertraline, 1S,4S-N-methyl-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthylamine, a new uptake inhibitor with selectivity for serotonin. J Pharmacol Exp Ther. 1983;226:686–700.


Redrobe JP, MacSweetney CP, Bourin M. The role of 5-HT₁₅ and 5-HT₁₈ receptors in antidepressant drug actions in the mouse
58 Takebayashi M, Hayashi T, Su TP. Nerve growth factor-induced neurite sprouting in PC12 cells involves σ1 receptors: implications for antidepressants. J Pharmacol Exp Ther. 2002;303:1227–1237.