Effects of sigma ligands on NMDA receptor function in the bulbectomy model of depression: a behavioural study in the rat

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Abstract

Sigma (σ) ligands have been shown to modulate NMDA receptor activity. In the present study we used the olfactory bulbectomy (OBX) animal model of depression to assess the effects of the σ₁ ligand igmesine on OBX-induced behaviour. Behavioural experiments demonstrated OBX (saline-treated) rats to have increased dizocilpine-induced behavioural modifications, including locomotor and circling activity as compared to Sham rats (saline-treated). A short-term (7 d) treatment with low doses of igmesine (50–200 µg/kg, d s.c.) had no effect on dizocilpine-induced behaviour while long-term treatments (14 d) with low doses of igmesine reversed the effect of the bulbectomy such that the treated OBX rats' behaviour was not significantly different from Sham-saline rats. Short-term treatments with high doses of igmesine (500–1000 µg/kg, d) also reversed the increased locomotor and circling behaviour seen in OBX rats (saline-treated) while long-term treatments with the same high doses did not. These results provide behavioural evidence for σ ligand's potential to reverse some OBX-induced behaviours. Moreover, they support the notion of a bell-shaped dose–response curve previously reported for σ ligands.

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Introduction

The existence of sigma (σ) receptors was first reported by Martin et al. (1976) who initially classified them as belonging to the opiate receptor family. Sigma receptors were later divided into 2 subtypes σ₁ and σ₂ based on their different ligand affinity, stereoselectivity, and response to various treatments (Itzhak and Stein, 1991; Quirion et al., 1992). In 1996, σ₁ receptors were cloned from guinea-pig liver, human placental cell line, mouse kidney and brain and rat brain (Hanner et al., 1996; Kekuda et al., 1996; Pan et al., 1998). In recent years, using an in vivo electrophysiological paradigm of unitary extracellular recordings from pyramidal neurons of the CA₁ or CA₂ region of the dorsal hippocampus, we have shown that acute intravenous administration of low doses of several high-affinity σ agonists, including igmesine (JO-1784) and (+)-pentazocine, does not affect the spontaneous firing activity of CA₁ pyramidal neurons, but produces a marked and selective dose-dependent potentiation of NMDA-induced firing activity (Bergeron et al., 1993; Monnet et al., 1990, 1992). Sigma ligands such as haloperidol, NE-100 and progesterone act as antagonists, not by modifying NMDA-induced firing activity, but by preventing and reversing the effects of the above-mentioned σ agonists (Monnet et al., 1990, 1992).

The majority of data implicating the σ receptor's role in depression involves the σ₁ subtype. Several σ₁ ligands (e.g. SA-4503, (+)-pentazocine, DTG, igmesine, and OPC-14523) have been shown to have antidepressant abilities in behavioural tests for antidepressants including the forced swimming test and tail suspension test, with NE-100, a selective σ₁ antagonist, blocking this effect (Kinsora et al., 1998; Matsumo et al., 1996; Tottori et al., 1997; Ukai et al., 1998). Moreover, preliminary results in a clinical trial suggest that igmesine might have antidepressant properties (Pande et al., 1998).

Olfactory bulbectomy (OBX) is currently recognized as a valuable animal model of major depression and useful in the study of the mechanisms of action of antidepressant drugs (Jesberger and Richardson, 1988; Kelly et al., 1997; Lumia et al., 1992). OBX in rodents provokes a variety of neurochemical and behavioural alterations, which are not related to anosmia and are reversible by a wide range of antidepressants (Grecksch et al., 1997; Jesberger and
Richardson, 1985; Kelly et al., 1997; Leonard and Tuite, 1981; Lumia et al., 1992; van Riezen and Leonard, 1990). It is thought to represent the neurochemical actions of antidepressants on depressive substrates relative to their actions on normal substrates (reviewed in Jesberger and Richardson, 1986). The behavioural changes induced by OBX appear 2–3 wk after the surgery and are characterized by hyperactivity, irritability, disruption of sexual behaviour (reviewed in Leonard and Tuite, 1981), deficits in learning avoidance responses, spatial memory (Archer et al., 1984) and sleep disturbances (Sakurada and Kisara, 1977; Sakurada et al., 1976). Furthermore, the measurable behavioural and biochemical alterations are normalized by chronic, but not acute administration of clinically efficacious antidepressant drugs from a variety of families (Jesberger and Richardson, 1986).

Indeed, previous studies in our laboratory have shown that OBX induces a down-regulation of NMDA receptors as, following OBX, the hyperactivity induced by the acute administration of the non-competitive NMDA antagonist dizocilpine was markedly decreased (Robichaud et al., 2001). In keeping with this finding, within 1 wk following OBX, $^{125}$Iiodo-dizocilpine binding was decreased in the frontal and piriform cortices, in the anteroventral thalamic nucleus and in certain amygdaloid nuclei, whereas, after 3 wk, this binding was also decreased in the postero medial cortex, the hippocampus and the lateral hypothalamus (Robichaud et al., 2001). The present study investigates the effects of short- and long-term treatments with the $\sigma_1$ agonist igmesine on dizocilpine-induced locomotor activity in OBX rats.

**Method**

Male Sprague–Dawley rats (180–275 g) were used. Animals were housed in temperature- (25 °C) and humidity-controlled rooms with a 12 h light/dark cycle (lights on at 07:00 hours) with food and water ad libitum. Rats were allowed 48 h of adaptation before undergoing surgery. Ethical Committee approval was given by the McGill University Animal Ethical Care Committee and all their rules and regulations were followed.

**Surgery**

Six groups of animals were studied. Three groups underwent OBX surgery, while three groups were Sham-operated. For OBX surgery (Jesberger and Richardson, 1986), animals were anaesthetized (chloral hydrate, 400 mg/kg i.p.) and fixed in a stereotaxic frame. Bilateral burr holes were made in the skull surface at the following coordinates: A, +5 mm (from Bregma); L, ± 2 mm. Olfactory bulbs were sectioned and removed by aspiration; the cavities being filled with haemostatic sponges. For Sham surgery, animals were similarly operated on but the bulbs were left intact. Following surgery animals were given 2 wk to recover and to permit the appearance of the 'OBX syndrome'. Two weeks after lesion, OBX and Sham-operated rats were randomly assigned to one of the five pharmacological treatments (saline, 50, 100, 200, 500 or 1000 µg/kg i.p. igmesine) for either 7 or 14 d. Drugs and saline were administered via an Alzet osmotic minipump (Alza, CA) inserted subcutaneously under halothane anaesthesia and aseptic conditions.

**Behavioural experiments**

All animals (Sham-operated and OBX rats) received a single injection of dizocilpine (200 µg/kg, i.p.). Animals were then placed in 80 cm diameter, circular activity-measuring wooden boxes with a 45 cm high wooden wall. Floors and walls were painted black and the only lighting was indirect and provided by a 40 W bulb located 2 m from the box. Locomotor activity was recorded with a video-tracking system (Videotrack, France) for 4-min time periods up to 40 min, encompassing the time-course for the maximal behavioural effects of dizocilpine (Löschler and Höнак, 1992). The different other behaviours measured: circling, head weaving and falling over were measured manually. Rectal temperature was also measured at 5 and 45 min. For assessing the effect of the acute administration of dizocilpine on locomotor activity, the mean ambulatory distance per minute was compared for each 4-min period from time 0 to time 40 min. As the pattern of changes in locomotion were similar in Sham and OBX animals (Figure 1), for...
assessing the effects of the short- and long-term treatments with igmesine, the mean value of the ambulatory distance per minute during the whole periods of 40 min were compared.

Drugs

Dizocilpine was purchased from Research Biochemicals International (Natick, MA, USA), igmesine was a generous gift from F. Roman (Institut de Recherche Jouvenal, Fresnes, France).

Histological verifications

Following behavioural experiments, the rats were sacrificed and all surgical procedures were verified. If any residual tissue of the main olfactory bulbs remained or if the frontal cortex had been damaged during the surgical procedures, then the behavioural data were not included in the final analysis.

Results

Exploratory behaviour

Following their introduction in the open field, the rats presented the usual exploratory behaviour, which progressively disappeared within 5–10 min. In the Sham-operated animals, the effects of the i.p. administration of dizocilpine (200 µg/kg) appeared within 10–15 min. The first manifestation was a progressive increase in locomotion followed by the appearance of stereotypies, head weaving, and circling behaviour and a marked decrease of rearing behaviour (Table 1, Figures 1, 2). Approximately 30 min following the administration of dizocilpine, the maximal behavioural effects were observed, the increased locomotion was generally reduced after that time since

Table 1. Behaviours observed before and after the acute administration of MK-801 (200 µg/kg i.p.)

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Before MK-801 (200 µg/kg i.p.)</th>
<th>After MK-801 (200 µg/kg i.p.)</th>
<th>No. of rats</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rearing</td>
<td>6.12 ± 0.93</td>
<td>0.25 ± 0.15</td>
<td>16</td>
<td>0.00001</td>
</tr>
<tr>
<td>Head weaving</td>
<td>0 ± 0</td>
<td>5.88 ± 1.38</td>
<td>16</td>
<td>0.0007</td>
</tr>
<tr>
<td>Circling</td>
<td>1.94 ± 0.31</td>
<td>6.01 ± 1.49</td>
<td>16</td>
<td>0.01</td>
</tr>
<tr>
<td>Falling over</td>
<td>0 ± 0</td>
<td>1.48 ± 0.51</td>
<td>16</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Numbers represent the mean ± standard error, p value according to Student’s t test. Post-injection observation period lasted from t + 8 to t + 40 min.

Figure 2. Mean (± S.E.M.) number of circling behaviours observed in OBX-saline rats (light bars) vs. Sham-saline rats (dark bars) following the acute injection of dizocilpine (200 µg/kg i.p.) administered at time 0. * p < 0.05 Student’s t test. Experiments were carried out in 14 OBX rats and 16 Sham rats.

Figure 3. Mean (± S.E.M.) number of (a) head weavings or (b) falling over behaviours recorded in Sham-saline vs. OBX-saline rats observed for a period of 20 min, starting 8 min following the injection of dizocilpine (200 µg/kg). * p < 0.05 Student’s t test. In this and the following figures the numbers at the foot of the columns indicate the number of rats observed.
Figure 4. Ambulatory distance travelled by Sham-saline, OBX-saline and OBX rats treated for (a) 7 d or (b) 14 d with igmesine (JO-1784). Values represent the mean (± s.e.m.) distance expressed in cm/min measured during the 40 min duration of the experiment, following dizocilpine injection (200 µg/kg). * p < 0.05 Student’s t test vs. Sham-saline.

Figure 5. Mean (± s.e.m.) number of circling behaviours observed in Sham-saline, OBX-saline, or OBX rats treated with 50–200 µg/kg.d of igmesine (JO-1784) for 14 d, following the acute administration of dizocilpine (200 µg/kg i.p.) at time 0. * p < 0.05 Student’s t test vs. Sham-saline.

Figure 6. Mean (± s.e.m.) number of (a) head weavings or (b) falling over behaviours recorded in OBX-saline, or OBX rats treated with 200 µg/kg.d of igmesine (JO-1784) for 14 d. Rats were observed for a period of 20 min, starting 8 min following the injection of dizocilpine (200 µg/kg i.p.). * p < 0.05 Student’s t test.

Effects of olfactory bullectomy

OBX rats treated with saline (OBX-saline) had significantly increased locomotor activity vs. Sham rats treated with saline (Sham-saline) in the 4–40 min period following the injection of dizocilpine (Figure 1). In OBX-saline rats, the behavioural effects of dizocilpine measured from 20 to 40 min following its administration, showed a greater than 30% increase in locomotor activity as measured by the distance travelled per minute (Figure 1). The grooming activity was not significantly changed by the injection of dizocilpine nor by the bullectomy (data not shown). Circling behaviour was increased in OBX-saline vs. Sham-saline rats, even if the difference was statistically significant only during the first 20 min (Figure 2). In contrast, head weaving and falling over (Figure 3) were markedly reduced (between 50 and 80%) following OBX.
Effect of sigma ligands on the olfactory bulbectomy model

Figure 7. Ambulatory distance travelled by Sham-saline, OBX-saline, or OBX rats treated with 500 or 1000 µg/kg.d of igmesine (JO-1784) for (a) 7 d or (b) 14 d. Values represent the mean (± s.e.m.) distance expressed in cm/min measured during the 40 min duration of the experiments, following injection of dizocilpine (200 µg/kg i.p.). *p < 0.05 Student’s t test vs. Sham-saline.

Effects of low doses of igmesine

Short-term (7-d) treatments of OBX rats with a low dose (200 µg/kg.d) of igmesine failed to produce any effect on the dizocilpine-induced locomotor behaviour (Figure 4a). However, in OBX rats, long-term treatments (14 d) with low doses of igmesine (50–200 µg/kg.d) dose-dependently decreased dizocilpine-induced motor effects in OBX rats (Figure 4b). Long-term treatments with low doses of igmesine also markedly decreased the circling behaviour in OBX rats vs. OBX-saline rats (Figure 5). In addition, long-term treatments with low doses of igmesine reversed the decrease in head-weaving behaviour seen in OBX-saline rats (Figure 6a). In contrast, 14-d treatment with igmesine (200 µg/kg.d) did not reverse the decrease in falling over seen in OBX compared to Sham-saline rats (Figure 6b).

Effects of high doses of igmesine

Short-term treatments with igmesine at higher doses (500–1000 µg/kg.d) induced a dose-dependent decrease in locomotor activity after dizocilpine injection in OBX rats, and therefore, a normalized response to dizocilpine vs. OBX-saline rats, comparable to Sham-saline-treated rats (Figure 7a). Long-term treatment with igmesine in high doses (500–1000 µg/kg.d) produced no significant difference between OBX saline- and OBX igmesine-treated rats regarding dizocilpine-induced behavioural modifications (Figure 7b). In agreement with these data, when circling behaviour was assessed, no effect of igmesine could be observed with long-term treatments with high doses (500 and 1000 µg/kg.d) (Figure 8). In Sham-operated animals treated for 2 wk with either low or high doses of igmesine, the locomotor and circling behaviour (data not shown) induced by dizocilpine, were
Figure 9. Ambulatory distance travelled by Sham-saline, OBX-saline and Sham rats treated with igmesine (JO-1784) for (a) 7 d or (b) 14 d. Values represent the mean (± S.E.M.) distance expressed in cm/min measured during the 40 min duration of the experiments, following the injection of dizocilpine (200 µg/kg i.p.).

not significantly different from those obtained in Sham-saline rats and thus were decreased vs. OBX-saline rats (Figure 9a, b).

Discussion

Five weeks after a bilateral OBX, and following 3 wk of saline treatment, the behavioural modifications such as ambulatory distance and circling behaviour induced by the acute administration of 200 µg/kg i.p. of dizocilpine were drastically increased in OBX-saline compared to Sham-saline rats (Figures 1, 2). In contrast, head weaving decreased in OBX-saline rats vs. Sham-saline rats. The hyper-locomotion induced by OBX as well as the marked behavioural effects and stereotypies induced by the acute administration of a low dose of dizocilpine are in keeping with previous studies (Deutsch and Hitri, 1993). The potentiation of the behavioural effects induced by the acute administration of dizocilpine is in agreement with the previous results of Redmond et al. (1997) who showed that acute treatment with dizocilpine (300 µg/kg) produced increased home cage locomotor activity while a lesser dose (100 µg/kg) attenuated home cage locomotor activity. Though this is a different setting than the ‘open field’ used in the present study, these results do correspond to the increase in locomotor activity seen in the present study in the ‘open field’ as we administered a dose of 200 µg/kg. Furthermore, chronic treatments with dizocilpine decreased the locomotor activity produced by OBX. This does not go against our observation as we administered only acute doses and the chronic dose could have different effects due to changes in receptor function that would not occur in our paradigm with the acute doses. As both the administration of dizocilpine and the OBX surgery alone increase locomotor activity, it is not surprising to find that their combined effect further increases locomotor activity.

The 2-wk treatment of OBX rats with low doses of igmesine (50–200 µg/kg,d), induced a reversal of the behavioural response to the injection of dizocilpine compared to what was observed in OBX-saline rats (Figure 4b). However, the short-term treatment with the same low doses did not lead to any significant difference in the dizocilpine-induced behavioural response compared to OBX-saline (Figure 4a). Conversely, a short-term treatment with the high doses of igmesine reversed the behavioural modifications induced by OBX, whereas a long-term treatment with the same doses was without any effect (Figure 7a, b).

We have previously reported that the dose–response curves of the potentiation of the NMDA response by σ ligands has a bell-shaped aspect (Bergeron et al., 1995). More specifically, following an acute intravenous administration, the maximum potentiation of the NMDA response was observed with 50 µg/kg of (+)-pentazocine and 4 µg/kg of igmesine (Bergeron et al., 1995). When higher doses were administered, the potentiating effect would progressively decrease and finally disappear and at higher doses, σ agonists were acting purely as σ antagonists (Bergeron et al., 1995). Moreover, we have shown that long-term treatments with low doses of σ agonists induces an up-regulation of σ receptors, but following a 3-wk treatment with low doses of DTG, (+)-pentazocine or igmesine, the neuronal activation induced by microiontophoretic applications of NMDA is markedly increased (Bergeron and Debonnel, 1997). Finally, it has been well established that long-term treatments with
antagonists induce a down-regulation of σ receptors (Bergeron and Debonnel, 1997; Itzhak and Alerhand, 1989; Jansen et al., 1992; Riva and Creese, 1990).

Therefore, during a long-term treatment with σ agonists a certain accumulation is needed in order for the ligands to function in the active ‘agonist range’. As the concentration of the ligand increases, its agonist effects increase as well as the sensitivity of the σ receptor. This continues until a peak after which the ligand begins to function as an antagonist and the effects of the ligand progressively decrease as the concentration increases and the σ receptor desensitizes. Thus, in the present study it is likely that at the lower dose the longer treatment was necessary in order for the igmesine to reach the ‘agonist range’. This explains why a shorter duration of treatment with a higher dose produced the same effects. Furthermore, the long-term treatment at high doses no longer produced any effect most likely due to the concentration of igmesine being in the ‘antagonist range’. This dose–response curve thus involves a change in the properties of the σ receptor and modify the NMDA response without affecting the sensitivity of the dizocilpine-binding site.

Furthermore, igmesine is a highly selective σ ligand, which has negligible affinity for other receptor subtypes including PCP, adrenergic, dopaminergic and serotonergic receptors (Roman et al., 1990), therefore, the effects observed in the present study are not likely mediated through activation of another receptor. Indeed, the present results suggest that, following a short-term treatment with a high dose and a long-term treatment with a low dose, igmesine is acting as an agonist and potentiates the response induced by the endogenous ligand for the still functional NMDA receptor. Therefore, in these conditions, it can be postulated that a chronic treatment with low doses of igmesine, by potentiating the effects of the down-regulated NMDA receptors produced by surgery in OBX, will compensate for this down-regulation, thereby normalizing the response to dizocilpine, and thus inducing smaller behavioural effects (Figures 4b, 5, 7a).

Recent studies have demonstrated interactions between several antidepressant drugs and the NMDA receptor complex. For example, the acute administration of desipramine, imipramine and nortriptyline drastically reduces NMDA-induced epileptiform response and long-term potentiation (LTP) in rat hippocampal slices (Watanabe et al., 1993), whereas a 5-wk treatment with desipramine, imipramine and amitriptyline inhibits the binding of [3H]dizocilpine in a concentration-dependent manner (Kitamura et al., 1991). Acute and chronic treatments with imipramine, amitriptyline, citalopram and fluoxetine potentiate the hyperactive behaviour induced by dizocilpine, an effect which is blocked by haloperidol, but not by the D1 and the D2 selective antagonists SCH-23390 or sulpiride, respectively (Maj et al., 1991, 1992), suggesting that the effect of haloperidol is likely due to its affinity for σ receptors and not for dopamine receptors. It has also been reported that chronic treatments with fluoxetine or imipramine reduce [3H](+)-pentazocine binding in the rat brain (Shirayama et al., 1993). In addition, several antidepressants have been found to decrease NMDA-activated ion current (Sernagor et al., 1989; White et al., 1990).

Swim stress and exposure to unpredictable mild stress increase the potency of glycine to displace [3H]5,7-dichlorokynurenic acid (DCKA) from the glycine site on the NMDA receptor in an imipramine-reversible manner (Nowak et al., 1995). This was specific to antidepressants after repeated treatments as structurally related non-antidepressant molecules did not produce this effect (Nowak et al., 1995). Similarly, adaptive changes of the NMDA receptor have been observed to occur selectively in the mouse cortex, where chronic SSRI treatments also decreased glycine-displaceable binding (Nowak et al., 1996, 1998). These data further strengthen the hypothesis that NMDA receptor modification could represent the final pathway of antidepressant action as already suggested by several groups (Paul et al., 1994; Skolnick et al., 1996, 1998). These data further strengthen the hypothesis that NMDA receptor modification could represent the final pathway of antidepressant action as already suggested by several groups (Paul et al., 1994; Skolnick et al., 1996). Combined with our experimental data, these different observations suggest that the NMDA and σ receptors may be involved in the pathophysiology of depression and in the mechanism of action of antidepressant treatments. Thus, a σ ligand that modulates NMDA receptor function could be expected to have some potential interest as an antidepressant treatment.

The inability of igmesine to reverse the dizocilpine-induced head weaving and falling over in OBX rats (Figure 6a, b) is likely due to these behaviours being mediated by pathways different from those involved in ambulation or circling behaviour. We have previously reported that following OBX, the modifications of NMDA-binding parameters differ in several brain regions (Robichaud et al., 2001). It is therefore plausible that some areas are either less enriched in σ receptors or have sustained greater modifications of NMDA-binding parameters which cannot be compensated for. This could also explain why the different responses to OBX surgery, such as head weaving and falling over are decreased in OBX rats (Figure 3a, b). Nonetheless, our results showed OBX to decrease MK-801-induced ataxia (falling over) (Figure 3b). Following treatments with igmesine (200 µg/kg), there was a further decrease in ataxia in addition to an increase in locomotor activity (Figure 6b). Therefore, this increase in locomotor activity could, in part, be due to the decrease in ataxia.
The effects observed in the present studies with igmesine may be due to the σ ligand's modulation of NMDA receptors previously discussed, however, a direct interaction with the serotonergic system cannot be ruled out. Recently, we have demonstrated the σ ligands (+)-pentazocine and 4-IBP to modulate serotonergic neurotransmission, as 2-d treatments (2 μg/kg, d) induced a 35% increase in average basal firing rate of the serotonergic neurons of the dorsal raphe nucleus, while igmesine at the same dose produced no change after short- or long-term treatments, using an electrophysiological paradigm of extracellular recordings in vivo (Bermack and Debonnel, 2001). Akunne et al. (2001) demonstrated that chronic treatment with igmesine (15 mg/kg, d) produced no change in 5-HT₁A receptor densities, only minor reductions in tyrosine hydroxylase activity, no effects on 5-HT, norepinephrine uptake nor 5-HT synthesis. Their study, in agreement with ours, suggested the pharmacological actions of igmesine may be in part due to mechanisms not mediated by the monoaminergic system and may involve NMDA receptors based on their observation of igmesine treatment blocking NMDA-induced increases in cGMP. This is in agreement with the published data mentioned by the reviewer showing 16-d treatment with igmesine (3 mg/kg) produced no change in serotonin turnover (Song et al., 1997).

Effective antidepressant treatments are expected to reverse OBX-induced alterations, thus normalizing NMDA receptor binding levels. Therefore, our results suggest that igmesine could have antidepressant properties in the OBX model. However, this would require further investigation comparing σ ligands to antidepressants with respect to various OBX-induced alterations. These experiments establish the first behavioural model indicating a behavioural effect of long-term treatment with low doses of σ ligands, most likely related to their affinity for σ receptors, reversing OBX-induced alterations.

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