In-vivo modulation of central 5-hydroxytryptamine (5-HT1A) receptor-mediated responses by the cholinergic system

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Abstract

The aim of the present study was to investigate a putative modulation of rat 5-HT system by the muscarinic receptor antagonist atropine using in-vivo electrophysiological and behavioural techniques. In the dorsal raphe nucleus, administration of atropine (1 mg/kg i.v.) prevented the suppressant effect of the selective serotonin reuptake inhibitor paroxetine (0.5 mg/kg i.v.) on the spontaneous firing activity of 5-HT neurons, suggesting that atropine could induce an attenuation of somatodendritic 5-HT1A autoreceptors responsiveness. The 5-HT1A receptor agonist 8-OH-DPAT decreased both immobility in the forced swim test and the body core temperature. Pre-treatment with atropine (5 and 10 mg/kg i.p.) enhanced antidepressant-like effect of 8-OH-DPAT (1 mg/kg s.c.) and reduced 8-OH-DPAT (0.1 mg/kg s.c.)-induced hypothermia. In conclusion, the present study reports a functional role of muscarinic receptors in the modulation of pre- and post-synaptic 5-HT1A receptors mediated responses.

Introduction

Although the pathophysiology of major depression and anxiety has not yet been elucidated, there is a growing body of evidence that supports the implication of the serotonin (5-HT) system in the therapeutic action of several classes of antidepressant drugs (Blier and de Montigny, 1999). In the dorsal raphe nucleus, which contains more than half of the brain 5-HT neurons, 5-HT neuronal firing activity is dependent not only on somatodendritic 5-HT1A autoreceptors but also on 5-HT1A receptors present on a long neuronal feedback loop originating from the forebrain (Piñeyro and Blier, 1999). Long-term treatment with 5-HT1A receptor agonists (Blier and de Montigny, 1987; Dong et al., 1997) or with a selective serotonin reuptake inhibitor (SSRI) (Blier et al., 1984; Jolas et al., 1994) reduces the inhibitory effect of 5-HT or of the 5-HT1A receptor agonist 8-OH-DPAT ejected by microiontophoresis onto 5-HT neurons, thus revealing a desensitization of somatodendritic 5-HT1A autoreceptors. The same treatments fail to modify the inhibitory effect of intravenously (i.v.) injected 8-OH-DPAT, suggesting the existence of post-synaptic 5-HT1A receptors regulating 5-HT neuronal activity and remaining unchanged after such chronic antidepressant treatments. However, some groups did find a decrease of the inhibitory effect of systemically administered 8-OH-DPAT on cortical 5-HT release (Dawson et al., 2002; Gartside et al., 2003). A discrete ablation of medial prefrontal cortex attenuated the 8-OH-DPAT-induced inhibition of 5-HT neurons to the same extent as cortical transection (Ceci et al., 1994; Hajos et al., 1998). Nevertheless, the neurochemical nature of this long loop remains to be fully specified, albeit the involvement of glutamatergic and GABAAergic neurons has been suggested but still needs to be further investigated (Celada et al., 2001; Haddjeri et al., 2000;
Haddjeri et al., 1999). Another neurotransmitter potentially involved in this neuronal circuit could be acetylcholine. In fact, systemic administration of 8-OH-DPAT increased acetylcholine output in frontal cortex of awake rats, and this effect of 8-OH-DPAT was prevented by the 5-HT1A receptor antagonist WAY-100635, but not by lesioning raphe 5-HT neurons (Consolo et al., 1996; Somboonthum et al., 1997). Similarly, 8-OH-DPAT, locally applied in the hippocampus via dialysis probes, enhances the release of acetylcholine (Nakai et al., 1998). In a previous in-vivo electrophysiological study, we have shown that the muscarinic receptor antagonists atropine and scopolamine reduced the suppressant effect of 8-OH-DPAT on the spontaneous firing activity of rat dorsal raphe 5-HT neurons (Haddjeri et al., 2000). Moreover, lesions of cholinergic neurons through the administration of AF-64A, directly in the medial prefrontal cortex or after its intracerebroventricular (i.c.v.) injection, attenuated the effect of 8-OH-DPAT. Thus, a key role for the glutamatergic, GABAergic and cholinergic systems on the feedback inhibition of dorsal raphe 5-HT neurons has been proposed (Celada et al., 2001; Haddjeri et al., 2000). Although the 5-HT hypothesis of depression seems to be well admitted, it is important to mention here that central cholinergic systems have been also proposed to play an important role in depressive disorders. For instance, stimulating central cholinergic neurotransmission can induce depressed mood, dysphoria or reduced hedonic capacity (Dilsaver, 1986; Janowsky and Overstreet, 1995; Mearns et al., 1994). Interestingly, the Flinders Sensitive Line (FSL) rats, that are more sensitive to direct-acting muscarinic agonist and have elevated muscarinic receptors, represent a good animal model of depression (Överstreet and Djuric, 2001).

Using in-vivo extracellular unitary recordings in anaesthetized rats, the aim of the present study was to further investigate a putative modulation of dorsal raphe 5-HT neurons by the muscarinic receptor antagonist atropine. Moreover, the hypothermic response to subcutaneous (s.c.) administration of the 5-HT1A receptor agonist 8-OH-DPAT and the capacity of cholinergic agents to modify this response was also examined. Finally, the possible modification of the antidepressant-like effect of 8-OH-PAT induced by atropine in the forced swimming test (FST) was explored.

Methods

The experiments were carried out in male Sprague-Dawley rats (Charles River, St Constant, Canada; Harlan, Gannat, France) weighing 250–300 g, which were kept under standard laboratory conditions (12:12 light–dark cycle with free access to food and water). Different groups of rats were used for the FST, the locomotor activity and the hypothermic responses. Before experiments, rats were kept in individual cages at stable temperature of 23 °C, for 1 h before the randomized experiments, in a room with the light already on in the afternoon. All animals were handled according to the guidelines approved by the Society of Neuroscience and all animal use procedures were approved by the Faculty Ethical Committee.

Extracellular recordings from dorsal raphe 5-HT neurons

Experiments were directly carried out under chloral hydrate anaesthesia (400 mg/kg i.p.). Unitary extracellular recordings of 5-HT neurons were performed with single-barrelled glass micropipettes. The tip was broken back to 2–4 μm and filled with a 2 M NaCl solution saturated with Fast Green FCF or Blue Chicago. Dorsal raphe 5-HT neurons were encountered over a distance of 1 mm starting immediately below the ventral border of the Sylvius aqueduct. These neurons present a slow (0.5–2.5 Hz) and regular firing rate and long duration (0.8–1.2 ms) positive action potentials (Aghajanian, 1978). The suppressant effect of the SSRI paroxetine (ED50 = 0.5 mg/kg i.v.; Hajos et al., 1995) on the firing activity of 5-HT neurons was assessed before and after the i.v. injection of atropine (1 mg/kg; Aghajanian et al., 1970).

Hypothermic responses

Body temperature was measured by inserting a lubricated probe 3 cm into the rectum while lightly restrained. The readings were obtained with a digital thermometer (TM99A, Harvard apparatus, South Natick, MA, USA). Two readings of the temperature were obtained immediately prior to the s.c. injection of 8-OH-DPAT (0.1 mg/kg) and one body core temperature was measured 30 min after 8-OH-DPAT injection. The dose of 8-OH-DPAT, atropine, scopolamine and the times of temperature measurements were based on previous dose- and time-dependent curves (Forster et al., 1995; Hjorth, 1985; Ryan et al., 1996). Atropine (10 mg/kg i.p.) and scopolamine (10 mg/kg i.p.) were injected 15 min after 8-OH-DPAT. Finally, 8-OH-DPAT-induced hypothermia was assessed in acetylcholine-lesioned rats. The lesions were performed by i.c.v. injecting the selective cholinergic toxin ethylcholine mustardaziridinium ion (AF-64A, 3 nmol in
2 μl per side at rate of 1 μl/min) under chloral hydrate anesthesia. The animals were tested 10 d later.

**FST**

We used the FST as previously described (Forsolt et al., 1977). Briefly, the rats experienced a pretest session followed 24 h later by a test session. For both the pretest and the test sessions, conducted under low illumination (12 lux), the rats were placed in a plastic cylindrical tank (50 cm high by 20 cm in diameter) filled with water at 22 ± 2 °C, with a depth of 40 cm, for which the hind limbs could not reach the tank floor. In all experiments, the pretest was carried out for 15 min and the test for 6 min in the same tank. Saline, 8-OH-DPAT (1 mg/kg s.c.) or atropine (5 mg/kg s.c.; Mancinelli et al., 1988) were administered alone or concomitantly between these two sessions (23 h, 5 h, and 1 h prior to the test sessions). Following either pretest 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 the animal’s behaviour online 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 approx. 95% of the corresponding frames classified as immobile for a non-swimming rat in its water tank. The same threshold was kept constant for naive as well as treated animals that were tested in a fixed and controlled lighting (12 lux). Software from ViewPoint permitted us to analyse data offline avoiding observer subjectivity (Malleret et al., 1999; Shearman et al., 2003).

**Locomotor activity**

These experiments were performed in order to ensure that the decreased immobility or the increased active behaviours in the FST were not secondary to a non-specific increase in locomotor activity produced by the treatments. Each drug, alone or in combination, was tested and animals receiving drugs were injected three times within 24 h (23 h, 5 h, and 1 h prior to session) in order to simulate the treatment procedures used in the FST (Mancinelli et al., 1988; Sousa et al., 2001). Rats were placed in activity cages which have photoelectric cells inserted allowing recordings of locomotor activity, i.e. quantification of the total number of activity counts (photocell beam breaks) during a 10-min session.

**Drugs**

Atropine sulphate, scopolamine hydrochloride, WAY-106635 trihydrochloride (N-[2-[4(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexane-carboxamide trihydroxychloride, 8-OH-DPAT hydrobromide [8-hydroxy-2-(dipropylamino)tetraline], AF-64A (acetyl ethylcholine mustard hydrochloride) were obtained from Research Biochemicals (Natick, MA, USA) or Sigma–Aldrich (St-Quentin Fallavier, France) and paroxetine from GSK (Harlow, UK). All drugs were dissolved in water, except AF-64A, which was dissolved in a Ringer solution. The concentrations, and the doses used for these compounds were chosen on the basis of previous successful experiments carried out in our, and other, laboratories.

**Data analysis**

Data are presented as mean ± S.E.M. In the electrophysiological, FST and locomotor activity experiments, statistical differences between the groups were analysed with two-way ANOVA followed by the post-hoc Student–Newman–Keuls test. For the hypothermia experiments, statistical differences between the groups were analysed with one-way ANOVA followed by the post-hoc Dunnett’s test. A $p$ value of $<0.05$ was considered significant.

**Results**

**Effects of atropine and paroxetine on the firing activity of dorsal raphe 5-HT neurons**

As illustrated in Figure 1b, atropine (1 mg/kg i.v.) by itself did not modify the firing activity of dorsal raphe 5-HT neurons. Without atropine (1 mg/kg i.v., Figure 1a), the mean firing activity of 5-HT neurons was 1.16 ± 1.9 Hz before the administration of 500 μg/kg i.v. of the SSRI paroxetine and 0 Hz after paroxetine injection ($n=6$). Interestingly, after atropine administration, the firing rate was $1.41 ± 1.8$ Hz before and $0.96 ± 1.9$ Hz after paroxetine injection ($n=7$, Figure 1b). Atropine significantly prevented the suppressant effect of paroxetine on the firing activity of 5-HT neurons as indicated by the results of the ANOVA $[F(1, 11)=14.9, p<0.01]$ (Figure 1c).

**Effects of 8-OH-DPAT and cholinergic ligands on the body temperature responses**

The distribution of the values from body temperature responses passed normality (Kolmogorov–Smirnov). As represented in Figure 2, there was a significant decrease in body temperature recorded in control rats.
30 min after the 8-OH-DPAT challenge. There was a significant effect of the treatments used on the hypothermic response induced by 8-OH-DPAT, as indicated by the results of the ANOVA \( F(3, 25) = 13.1, p < 0.01 \). The post-hoc Dunnett’s test further revealed that both atropine and scopolamine \( p < 0.05 \) and AF-64A \( p < 0.01 \) groups were significantly different from the vehicle further suggesting the involvement of cholinergic and 5-HT neurons in this physiological response.

**Effect of atropine on the anti-immobility action of 8-OH-DPAT in the FST**

The distribution of the values from FST passed normality (Kolmogorov–Smirnov) with the exception of the atropine group in the last case. However, this latter failure was very close to normality \( (p = 0.0491) \) and we have assumed a pseudo-parametric distribution in this case, and performed parametric tests. Figure 3 shows the effect of saline, 8-OH-DPAT (1 mg/kg s.c.), atropine (5 mg/kg s.c.) and atropine + 8-OH-DPAT combination on the total time of immobility behaviour evaluated in the FST. 8-OH-DPAT and atropine significantly reduced immobility behaviour by 31 and 33% respectively (two-way ANOVA \( F(1, 61) = 13.3, p < 0.001 \) and \( F(1, 61) = 11.7, p < 0.001 \) when considering the ‘pre-treatment’ and ‘treatment’ factors respectively). Importantly, atropine facilitated the

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**Figure 1.** Integrated firing rate histograms of 5-HT neurons recorded in the dorsal raphe nucleus showing their responses to (a) paroxetine and WAY-100635, and (b) atropine and paroxetine. Mean results \( \pm \text{S.E.M.} \) of the suppressant effect of paroxetine on the firing activity of 5-HT neurons in control rats or after the i.v. administration of atropine (1 mg/kg) is represented in (c). The numbers at the bottom of the columns indicate the number of rats tested. **\( p < 0.01 \): vehicle vs. atropine using repeated ANOVA followed by Student–Newman–Keuls test (see Results section).

**Figure 2.** Hypothermic response to s.c. injection of 8-OH-DPAT alone (0.1 mg/kg s.c., \( n = 6 \)) and prior to i.p. administration of atropine (10 mg/kg s.c., \( n = 7 \)) and scopolamine (0.1 mg/kg s.c., \( n = 7 \)) as well as in AF-64A-lesioned rats (injected i.c.v., \( n = 6 \)). Baseline core-body temperatures, in degrees Centigrade, were measured as described in the Materials and methods section prior to and 30 min after the injection of 8-OH-DPAT. Values shown are the decrease \( \pm \text{S.E.M.} \). **\( p < 0.001 \) and * \( p < 0.05 \): using one-way ANOVA followed by Dunnett’s test.
antidepressant-like behaviour of 8-OH-DPAT in the FST (a decrease of 60% compared to saline). However, the interaction between 8-OH-DPAT and atropine was not significant \(F(1, 58) = 0.06, \text{n.s.}\], indicating that the two effects are additive. Indeed, the post-hoc Student–Newman–Keuls test reported that the atropine + 8-OH-DPAT group was significantly different from both the atropine and the 8-OH-DPAT ones \((p < 0.05\) in each case).

**Effects of 8-OH-DPAT and atropine on the locomotor activity**

The distribution of the values from locomotor activity passed normality (Kolmogorov–Smirnov). Figure 4 shows the results of the horizontal locomotor activity. At a dose of 5 mg/kg s.c., which induced antidepressant-like effects in the FST (Figure 3), atropine did not produce any significant change in the locomotor activity (decrease of 20% \(F(1, 28) = 0.3, \text{n.s.}\) when considering the ‘pre-treatment’ factor). Conversely, 8-OH-DPAT alone reduced this activity by 88% \(F(1, 28) = 13, p < 0.001\) when considering the ‘treatment’ factor, whereas the combination of 8-OH-DPAT with atropine decreased the locomotor activity by 60%. Also, the interaction between the two drugs was not significant \(F(1, 31) = 2.6, \text{n.s.}\).

**Discussion**

The present results show that the muscarinic receptor antagonist atropine (1) attenuated the suppressant effect of paroxetine on the spontaneous firing activity of rat dorsal raphe 5-HT neurons, (2) significantly reduced the 8-OH-DPAT-induced lowering body temperature (as it was the case with scopolamine and AF-64A pre-treatment) and (3) potentiated the reduction of immobility behaviour produced by 8-OH-DPAT in the FST.

As mentioned in the Introduction, 5-HT neuronal activity in the dorsal raphe nucleus is primarily regulated via 5-HT\(_{1A}\) autoreceptors (Lanfumey and Hamon, 2000) but is also dependent on 5-HT\(_{1A}\) receptors being present on a long neuronal feedback loop originating from the medial prefrontal cortex (Celada et al., 2001; Haddjeri et al., 2000; Hajos et al., 1999). In order to further investigate the modulation of 5-HT neuronal firing activity by muscarinic receptors (Haddjeri et al., 2000), the present study shows that i.v. administration of atropine significantly prevented the suppressant effect of paroxetine on the spontaneous firing activity of rat dorsal raphe 5-HT neurons. In contrast to the action of 8-OH-DPAT, a lesion of medial prefrontal cortex does not modify the suppressant effect of paroxetine on dorsal raphe 5-HT neuronal activity (Hajos et al., 1999) ruling out the possibility that paroxetine could mediate its effect through this long pathway. Although difficult to interpret in mechanistic terms, one possibility can be a direct interaction between 5-HT and cholinergic neurons. This hypothesis remains plausible since lesions of cholinergic neurons, induced by AF-64A, attenuate the inhibitory effect of 8-OH-DPAT (Haddjeri et al., 2000) and reduce 5-HT\(_{1A}\) receptor binding in raphe nuclei (Lim et al., 2000). Furthermore, 5-HT inhibits more than two-thirds of the laterodorsal tegmental neurons while half...
of dorsal raphe 5-HT neurons are inhibited by locally applied acetylcholine (Koyama and Kayama, 1993). Finally, laterodorsal tegmental cholinergic axon terminals and synapses as well as muscarinic receptors in the rat dorsal raphe nucleus have been also described (Baghdoyan, 1997; Wang et al., 2000). Further experiments, including those on putative pharmacokinetic and cardiovascular interactions, are needed to validate the role of the cholinergic nuclei in the effect of SSRIs such as paroxetine on dorsal raphe 5-HT neuronal firing activity that seems not to involve neural circuits connected with the medial prefrontal cortex (Hajos et al., 1999).

In basal condition, ours and other studies showed that rat body temperature is above 38°C (Cryan et al., 2000; Oerther and Ahlenius, 2001). On the other hand, core temperature is approx. 37-37.5°C when measured by telemetry (Nicholas and Seiden, 2003; Rowsey and Gordon, 2000) suggesting that rats are under stress in our conditions and that drugs could affect stress-induced hyperthermia. Taking this point into account, systemic administration of 8-OH-DPAT produces a dose-dependent hypothermia that can be prevented by pre-treatment with the 5-HT1A receptor antagonist WAY-100635 (Forster et al., 1995; Hjorth, 1985). Although a presynaptic component has been observed in rats (Hillegaart, 1991), this response seems to be mediated by an enhanced activation of post-synaptic 5-HT1A receptors. In particular, inhibition of 5-HT synthesis and the lesion of 5-HT neurons fail to prevent hypothermia-evoked response to systemic administration of 5-HT1A receptor agonists (Barnes and Sharp, 1999). Surprisingly in the present study, i.c.v. injection of AF-64A as well as a pre-treatment with atropine or scopolamine, significantly attenuated the 8-OH-DPAT-induced hypothermic response, suggesting a cross-link between cholinergic and 5-HT systems in this physiological response. In this regard, it has been shown that AF-64A administration decreases 5-HT transporter binding in the rat frontal cortex and hippocampus, reduces 5-HT1A receptor binding in raphe nuclei and hippocampal 5-HT content as well as increasing the tryptophan hydroxylase activity in the striatum and frontal cortex (Eva et al., 1987; Lim et al., 2000). The reasons of such modifications are still unknown. Conversely, our results are in accordance with the fact that 8-OH-DPAT-induced hypothermia is more pronounced in the FSL model, animals that exhibit supersensitive responses to cholinergic agonists (Overstreet et al., 1998).

Forced swimming is a behavioural test for rodents, which predicts the efficacy of antidepressant drugs (Lucki, 1997; Porsolt et al., 1977). Although a presynaptic component has been also proposed (Cervo and Samanin, 1987, 1991; Cervo et al., 1988; Matsuda et al., 1995), a role for post-synaptic 5-HT1A receptors in the immobility effects of 5-HT1A receptor ligands including 8-OH-DPAT, as well as of several antidepressants, has been suggested (Borsini, 1995; Detke et al., 1995; Lucki et al., 1994; Luscombe et al., 1993; Wieland and Lucki, 1990). In the present study, systemic administration of 8-OH-DPAT reduced immobility in the FST, thereby producing antidepressant-like behaviour in this model. The latter effect has been shown to be prevented by the 5-HT1A receptor antagonist WAY-100635 (Martinez-Mota et al., 2002; Moser and Sanger, 1999). The effect of atropine in the FST seems more complex due to its dose-dependent modification in locomotor activity (Mancinelli et al., 1988; Sousa et al., 2001). However, a contribution of anticholinergic effects to the action of antidepressants in the FST has been previously reported. It has been shown that the anti-immobility effect in the FST of desipramine and nomifensine is potentiated by atropine or scopolamine and antagonized by the acetylcholine esterase inhibitor physostigmine (Mancinelli et al., 1988). This group has suggested that the cholinergic system may control the neural circuitry upon which antidepressants act to reduce immobility time (Mancinelli et al., 1988). Cholinomimetics increase behavioural depression in the FST (Hasey and Hanin, 1991; Overstreet, 1986), whereas muscarinic antagonists can reduce this behaviour (Mancinelli et al., 1988; Overstreet, 1986). It has been proposed that muscarinic 1 (M1) receptors in the nucleus accumbens may be involved in the regulation of behavioural depression in the FST. Local injections of M1 receptor antagonists in this nucleus appeared to have an antidepressant effect (Chau et al., 2001). In the present study, both 8-OH-DPAT and atropine administration reduced immobility in the FST and such effects do not seem to be related to change in locomotor activity (see Figure 4). It remains difficult, however, to explain why and how atropine potentiates the anti-immobility action of 8-OH-DPAT. Nevertheless, studies characterizing modulations of the 5-HT system by muscarinic mediators may be important to relate here. For instance, Hery et al. (1977) found in vitro that the muscarinic agonist oxotremorine reduces the spontaneous release of newly synthesized [3H]5-HT in rat hypothalamus slices and such effect is prevented by both atropine and scopolamine. Interestingly, both atropine and scopolamine given alone increase [3H]5-HT release (Hery et al., 1977). Similarly, it has been more recently shown that the M1 receptor antagonist pirenzepine increases...
[1H]5-HT release in rat hippocampus slices (File et al., 2000). Conversely in vivo, both 8-OH-DPAT and atropine, locally applied in the hippocampus via dialysis probes, enhance the release of acetylcholine (Fujii et al., 1999; Nakai et al., 1998). Although still speculative, both adaptive changes at the presynaptic level combined with increases of 5-HT release at the terminal level can be taken into account for the potentiating action of atropine in the anti-immobility effect of 8-OH-DPAT.

In summary, the present results show that the muscarinic receptor antagonist atropine attenuated both the suppressant effect of paroxetine on the spontaneous firing activity of rat dorsal raphe 5-HT neurons, and 8-OH-DPAT-induced hypothermia. Moreover, atropine potentiated the reduction of immobility produced by 8-OH-DPAT as evaluated in the FST. Hence, the present studies unveil a key role for the cholinergic system in modulating 5-HT neuronal firing but not necessarily involving a long neuronal feedback loop. The present results also show that atropine is able to attenuate the responsiveness of somatodendritic as well as post-synaptic 5-HT1A receptors by mechanisms which remain to be elucidated.

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Statement of Interest

None.

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