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Increased waking after intra-accumbens injection of *m*-chlorophenylbiguanide: prevention with serotonin or dopamine receptor antagonists

Ana Ponzoni [a], Jaime M. Monti [a,*], Héctor Jantos [a], Humberto Altier [a], Daniel Monti [b]

[a] *Department of Pharmacology and Therapeutics, School of Medicine, Clinics Hospital, Montevideo 11600, Uruguay*
[b] *Department of Pharmacology, University of Illinois, College of Medicine, Chicago, IL 60612, USA*

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Abstract

Bilateral injection of the selective 5-HT_3 receptor agonist *m*-chlorophenylbiguanide (5.0–40.0 μg) into the nucleus accumbens of the rat significantly increased waking and decreased slow wave sleep. Rapid eye movement (REM) sleep remained unchanged. Pretreatment with the 5-HT_3 receptor antagonist MDL 72222 (1*aH*,3*a*,5*a*,*H*-tropan-3-yl-3,5-dichloro-benzoate) (0.5 mg/kg s.c.) reversed the effects of *m*-chlorophenylbiguanide (10.0–20.0 μg) on sleep and waking. Blockade of the dopamine D_1 or D_2 receptor with (+)-SCH 23390 (0.25 mg/kg s.c.) or YM-09151-2 (*cis*-*N*-(1-benzyl-2-methylpyrrolidin-3-yl)-5-chloro-2-methoxy-4-methylaminobenzamide) (0.5 mg/kg s.c.), respectively antagonized the increase of waking and reduction of slow wave sleep induced by *m*-chloro-phenylbiguanide (10.0 μg). Our results tend to indicate that the increase of wakefulness after injection of the selective 5-HT_3 receptor agonist *m*-chlorophenylbiguanide into the nucleus accumbens is partly related to the release of endogenous dopamine. In addition, they suggest that concomitant stimulation of both accumbens dopamine D_1 and D_2 receptor-related mechanisms is a necessary prerequisite to increase wakefulness.

Keywords: Sleep; Waking; *m*-Chlorophenylbiguanide; 5-HT_3 receptor; MDL 72222; Dopamine; (+)-SCH 23390; YM-09151-2

1. Introduction

Recently, we showed that the selective 5-HT_3 receptor agonist *m*-chlorophenylbiguanide injected into the lateral ventricle of the rat increased wakefulness and rapid eye movement (REM) sleep latency, whereas slow wave sleep, REM sleep and the number of REM periods were reduced. The highly selective and potent 5-HT_3 receptor antagonist MDL 72222 (1*aH*,3*a*,5*a*,*H*-tropan-3-yl-3,5-dichloro-benzoate) prevented the effects of *m*-chlorophenylbiguanide (Ponzoni et al., 1993). Concerning the mechanism underlying the *m*-chlorophenylbiguanide-induced increase of waking and decrease of sleep, it has been proposed that 5-HT_3 receptor agonists presumably act by increasing the release of several endogenous neurotransmitters, predominantly dopamine (Kilpatrick and Tyers, 1992; Tricklebank, 1992) which during a second step would enhance wakefulness (Monti et al., 1989).

Serotonergic neurons in the raphe system have been characterized which project to dopamine-containing cells in the ventral tegmental area and substantia nigra, and to the projection fields in the ventral and dorsal striatum (Steinbush, 1984; Hervé et al., 1987). The serotonergic pathway to the nucleus accumbens originates primarily in the caudal aspect of the dorsal raphe nucleus (Van Bockstaele et al., 1993).

Serotonin has been found to concentration-dependently increase dialysate dopamine when perfused in the nucleus accumbens of the rat (Parsons and Justice, 1993). Similar effects were obtained after direct application of the specific 5-HT_3 receptor agonist 1-phenylbiguanide into the nucleus accumbens. The action of 1-phenylbiguanide was not abolished by depleting forebrain serotonin with 5,7-dihydroxytryptamine, thus suggesting that the 5-HT_3 receptors involved in dopamine modulation are located on the presynaptic dopaminer-

* Corresponding author. Department of Pharmacology and Therapeutics, Clinics Hospital, J. Zudañez 2833/602, Montevideo 11300, Uruguay. Tel. 598 2 470782×472707, fax 598 2 473787.

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gic terminals in the nucleus accumbens (Chen et al., 1991).

The present study was designed to quantify the effect of direct application of the 5-HT_3 receptor agonist, *m*-chlorophenylbiguanide, into the nucleus accumbens on sleep and waking in the rat. In addition, we examined the potential use of MDL 72222 as 5-HT_3 receptor antagonist against *m*-chlorophenylbiguanide-induced changes in sleep variables.

Recent studies have shown that the highest values of dopamine D_1 and D_2 receptor densities in the rat are found in the basal ganglia and associated areas such as the nucleus accumbens (Camps et al., 1990), which prompted us to ascertain whether pretreatment with the dopamine D_1 receptor antagonist (+)-SCH 23390 or the dopamine D_2 receptor antagonist YM-09151-2 (*cis*-*N*-(1-benzyl-2-methylpyrrolidin-3-yl)-5-chloro-2-methoxy-4-methylaminobenzamide) would modify the actions of *m*-chlorophenylbiguanide on sleep variables.

2. Materials and methods

Male Wistar rats weighing 300–350 g were implanted with electrodes for chronic recording of electroencephalogram and electromyogram activities from the frontal and occipital cortex and from the dorsal neck musculature. In addition, stainless steel cannulae (25 gauge) were implanted bilaterally such that their tips were placed 1 mm above the nucleus accumbens (2.0 mm anterior to bregma, 1.5 mm lateral from the midline, and 6.6 mm below the top of the skull; coordinates according to Paxinos and Watson (1986). Drug or vehicle was injected into the nucleus accumbens with an injection cannula (31 gauge) extending 1 mm below the guide cannula. Only data from animals where histological inspection of their brains post-mortem demonstrated that the cannulae were within the limits of the intended target area were included in the presentation of the results.

The animals were housed individually in a temperature-controlled room (21 ± 1°C), under a 12 h light/12 h dark cycle (lights went on at 7.00 a.m.), and with food and water ad libitum. Ten days after surgery the animals were habituated to a soundproof chamber fitted with slip-rings and cable connectors. Thereafter, they were given either a control solution or the drug(s) to be tested. The electrographic activity of 50 s epochs was analised and assigned to the following categories, based on the waveform: wakefulness, light sleep, slow wave sleep and REM sleep (Monti et al., 1988). Slow wave sleep and REM sleep latencies and the number of REM periods were determined in addition.

We studied the effects of *m*-chlorophenylbiguanide hydrochloride (Cookson, UK) 5.0, 10.0, 20.0 and 40.0 μg as base injected into the nucleus accumbens. Drug or vehicle (saline) was infused bilaterally in a volume of 1 μl over a period of 60 s and the injection cannula was left in situ for another 60 s. Taking into consideration the size of the nucleus accumbens it seemed justified to administer drug or vehicle in a 1 μl volume. Moreover, in several studies where drugs were microinjected into the nucleus accumbens an experimental protocol similar to ours was followed (Wong et al.,

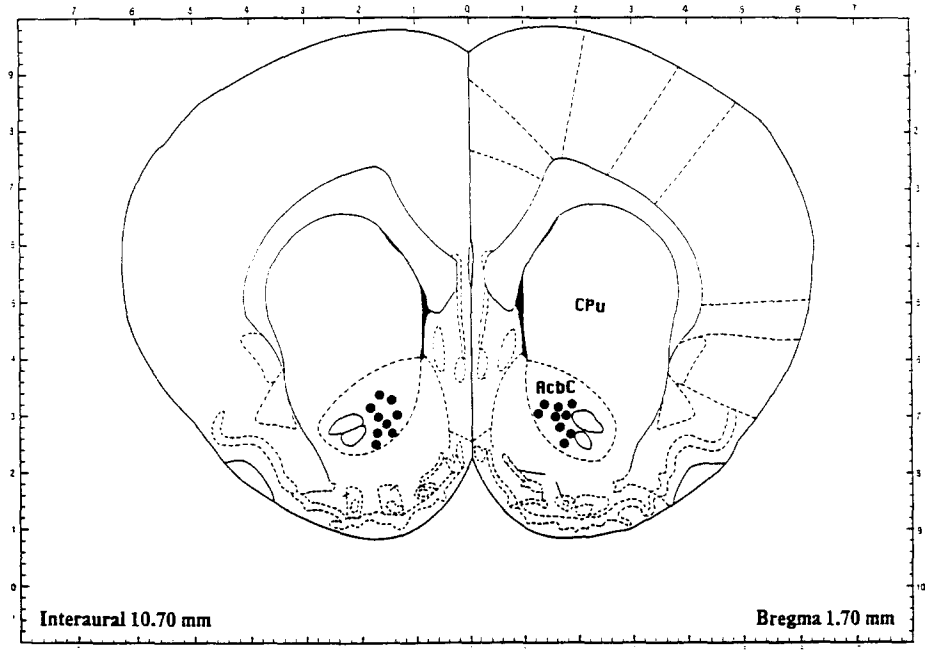

Fig. 1. Schematic drawing of injection sites in the nucleus accumbens (n = 9). Abbreviations: AcbC = nucleus accumbens; CPu = caudate-putamen. Section according to Paxinos and Watson (1986).

1991; Ahlenius, 1992; Johnson and Stellar, 1994). In the second set of experiments *m*-chlorophenylbiguanide 10.0 or 20.0 μg was injected into animals pretreated with MDL 72222 (Merrell Dow, USA) 0.5 mg/kg s.c. In the third set of experiments *m*-chlorophenylbiguanide 10.0 μg was injected into animals pretreated with YM-09151-2 (Yamanouchi, Japan) 0.5 mg/kg s.c. or (+)-SCH 23390 (RBI, USA) 0.25 mg/kg s.c. In the end, each rat was given seven microinjections into the nucleus accumbens. Doses of the 5-HT_3 receptor antagonist, and the dopamine D_1 or D_2 receptor antagonists given in the present study, were shown previously to effectively antagonize the effect of the corresponding agonists on sleep variables (Monti et al., 1989, 1990; Ponzoni et al., 1993). The drugs were given 15 min apart in the interaction experiments. MDL 72222 and YM-09151-2 were dissolved in a small volume of glacial acetic acid and diluted with distilled water; the pH was adjusted to 6.0. Subcutaneous injections were given in a final volume of 1.0 ml/kg. Immediately after injection into the nucleus accumbens and 15 min after s.c. injection a 6 h recording was started at approximately 8.30 a.m. At least 5 days were allowed to elapse between experiments to avoid long-lasting and rebound effects on sleep. One-way analysis of variance with multiple measures was used for statistical comparison of four or more samples, with multiple post-hoc comparisons performed by the Newman-Keuls test when the ANOVA was significant.

3. Results

The positioning of injections into the nucleus accumbens of the animals included in the experiments is shown schematically in Fig. 1. In two animals histological analysis showed a slight disruption of brain tissue surrounding the injection area. Nevertheless, values corresponding to sleep variables after the different treatments were within the range observed in the remaining animals.

Table 1
Effects of *m*-chlorophenylbiguanide injected into the accumbens nucleus on sleep and waking

	W	LS	SWS	REMS
0–3 h				
Control	46.2± 4.1	23.5±3.6	102.6±4.1	7.7±1.2
m-Chlorophenylbiguanide				
5.0 μg	55.8± 4.2	24.9±2.1	91.7±4.6	7.6±1.9
10.0 μg	65.7± 3.5a	25.9±4.1	83.7±4.2 [a]	4.7±1.2
20.0 μg	77.0± 6.4 [b]	22.1±2.6	76.9±5.7 [b]	4.0±1.9
40.0 μg	72.6±10.1 [b]	31.2±4.2	71.4±9.5 [c]	4.8±1.6
4–6 h				
Control	31.9± 3.0	29.2±3.6	104.9±4.9	14.0±1.0
m-Chlorophenylbiguanide				
5.0 μg	32.8± 4.3	30.4±2.4	105.9±4.5	10.9±1.8
10.0 μg	44.6± 5.0	34.2±4.2	89.7±6.5	11.5±2.3
20.0 μg	30.4± 4.7	26.0±3.7	110.6±7.0	13.0±2.2
40.0 μg	36.4± 8.0	35.6±5.1	96.9±8.8	11.1±2.5

W = wakefulness; LS = light sleep; SWS = slow wave sleep; REMS = REM sleep. Nine animals were in each experimental group. All values are the means (min)±S.E.M. The doses are in μg. Compared with control values: [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$ (Newman-Keuls test).

Table 2
Effects of pretreatment with MDL 72222 on the *m*-chlorophenylbiguanide-induced changes of sleep and waking

	W	LS	SWS	REMS
0–3 h				
Control	47.0±2.8	22.6±4.7	102.4±2.9	8.0±1.6
m-Chlorophenylbiguanide				
10.0 μg	66.7±3.7 [a]	21.3±4.3	85.4±5.1 [a]	6.6±1.4
20.0 μg	74.8±6.7 [b]	21.9±3.2	79.1±6.0 [b]	4.2±2.2
MDL 72222 0.5 mg/kg + *m*-chlorophenylbiguanide 10 μg	48.4±4.9	22.9±3.8	98.7±5.6	10.0±2.5
MDL 72222 0.5 mg/kg + *m*-chlorophenylbiguanide 20 μg	45.3±2.6	22.7±2.8	104.7±4.0	7.3±1.8

Seven animals were in each experimental group. All values are the means (min)±S.E.M. Compared with control values: [a] $P < 0.01$; [b] $P < 0.001$ (Newman-Keuls test).

Table 1 shows that bilateral intra-accumbens injection of *m*-chlorophenylbiguanide (5.0–40.0 μg) induced a significant increase of wakefulness and a reduction of slow wave sleep during the first three recording hours. As compared to control values light sleep, REM sleep, sleep latencies and the number of REM sleep periods showed no significant changes. Pretreatment with MDL 72222 (0.5 mg/kg) prevented the *m*-chlorophenylbiguanide-induced reduction of slow wave sleep and the increase of wakefulness (Table 2). Sleep latencies and the number of REM sleep periods were not significantly modified.

Treatment with YM-09151-2 (0.5 mg/kg) modified only slightly the amount of time spent in sleep or waking during the first three recording hours. YM-09151-2 effectively antagonized the increase of wakefulness and decrease in slow wave sleep induced by *m*-chlorophenylbiguanide (10 μg). In addition, REM

Table 3
Effects of pretreatment with YM-09151-2 on the *m*-chlorophenylbiguanide-induced changes of sleep and waking

	W	LS	SWS	REMS
0–3 h				
Control	47.0±2.8	22.6±4.7	102.4± 2.9	8.0±1.6
m-Chlorophenylbiguanide 10 μg	66.7±3.7 [b]	21.3±4.3	85.4± 5.1 [a]	6.6±1.4
YM-09151-2 0.5 mg/kg	38.3±4.2	28.7±2.9	107.0± 4.7	6.0±1.0
YM-09151-2 0.5 mg/kg + *m*-chlorophenylbiguanide 10 μg	35.2±5.5	38.2±6.3	103.9±10.7	2.7±0.6 [b]

Seven animals were in each experimental group. All values are the means (min)±S.E.M. Compared with control values: [a] $P < 0.05$; [b] $P < 0.01$ (Newman-Keuls test).

Table 4
Effects of *m*-chlorophenylbiguanide and YM-09151-2 pretreatment on sleep latencies and the number of REM periods

	Slow wave sleep latency (min)	REM sleep latency (min)	No. of REM periods 0–3 h
Control	20.6 ± 3.6	71.4 ± 19.4	4.0 ± 0.6
m-Chlorophenylbiguanide 10 μg			
	21.0 ± 3.3	104.1 ± 27.4	3.3 ± 0.8
YM-09151-2 0.5 mg/kg			
	11.0 ± 2.3	84.3 ± 18.9	2.9 ± 0.5
YM-09151-2 0.5 mg/kg + *m*-Chlorophenylbiguanide 10 μg			
	13.3 ± 3.5	132.9 ± 11.7	1.4 ± 0.3 [a]

All values are the means ± S.E.M. Seven animals were in each experimental group. Compared with control values: [a] $P < 0.01$ (Newman-Keuls test).

Table 5
Effects of pretreatment with (+)-SCH 23390 on the *m*-chlorophenylbiguanide-induced changes of sleep and waking

	W	LS	SWS	REMS
0–3 h				
Control	47.0 ± 2.8	22.6 ± 4.7	102.4 ± 2.9	8.0 ± 1.6
m-Chlorophenylbiguanide 10 μg				
	66.7 ± 3.7 [b]	21.3 ± 4.3	85.4 ± 5.1 [a]	6.6 ± 1.4
SCH 23390 0.25 mg/kg				
	36.2 ± 5.4	24.5 ± 4.2	108.7 ± 3.8	10.6 ± 1.3
SCH 23390 0.25 mg/kg + *m*-chlorophenylbiguanide 10 μg				
	26.3 ± 3.8 [a]	24.2 ± 6.4	119.2 ± 6.6a	10.3 ± 2.1

Seven animals were in each experimental group. All values are the means (min) ± S.E.M. Compared with control values: [a] $P < 0.01$; [b] $P < 0.001$ (Newman-Keuls test).

sleep and the number of REM periods were reduced (Tables 3 and 4).

Slow wave sleep latency was significantly decreased following treatment with 0.25 mg/kg (+)-SCH 23390. Pretreatment with (+)-SCH 23390 prevented the increase of wakefulness and reduction of slow wave sleep induced by *m*-chlorophenylbiguanide (10 μg). The combined treatment induced also a decrease of slow wave sleep latency (Tables 5 and 6).

Table 6
Effects of *m*-chlorophenylbiguanide and SCH 23390 pretreatment on sleep latencies and the number of REM periods

	Slow wave sleep latency (min)	REM sleep latency (min)	No. of REM periods 0–3 h
Control	20.6 ± 3.6	71.4 ± 19.4	4.0 ± 0.6
m-Chlorophenylbiguanide 10 μg			
	21.0 ± 3.3	104.1 ± 27.4	3.3 ± 0.8
SCH 23390 0.25 mg/kg			
	7.6 ± 3.1 [b]	63.6 ± 9.8	4.1 ± 0.6
SCH 23390 0.25 mg/kg + *m*-chlorophenylbiguanide 10 μg			
	11.1 ± 2.5 [a]	55.0 ± 11.0	4.7 ± 0.9

Seven animals were in each experimental group. All values are the means ± S.E.M. Compared with control values: [a] $P < 0.05$; [b] $P < 0.02$ (Newman-Keuls test).

4. Discussion

The major finding of the present study is that direct application of the specific 5-HT_3 receptor agonist *m*-chlorophenylbiguanide into the nucleus accumbens increases waking and reduces slow wave sleep without suppressing REM sleep. The effect of *m*-chlorophenylbiguanide on slow wave sleep and waking was prevented by prior administration of the selective 5-HT_3 receptor antagonist MDL 72222.

Effects on sleep after intra-accumbens administration of *m*-chlorophenylbiguanide differed from those observed following intracerebroventricular injection of the 5-HT_3 receptor agonist. Thus, the latter induced an increase of waking but reduced slow wave sleep, REM sleep and the number of REM periods (Ponzoni et al., 1993). REM sleep suppression after intracerebroventricular injection of *m*-chlorophenylbiguanide could be tentatively related to activation of 5-HT_3 receptors confined to areas critical in the generation of REM sleep, including the medial pontine reticular formation. However, further studies are needed to resolve this issue.

In vivo and in vitro studies have shown that 5-HT_3 receptor agonists regulate the release of dopamine, acetylcholine, noradrenaline, cholecystokinin and serotonin itself. The effects on dopamine, cholecystokinin and serotonin release are excitatory; all are reversed by 5-HT_3 receptor antagonists (Jiang et al., 1990; Chen et al., 1991; Kilpatrick and Tyers, 1992).

A serotonergic pathway to the nucleus accumbens which originates primarily in the caudal aspect of the dorsal raphe nucleus has been described (Van Bockstaele et al., 1993). In addition, high levels of the 5-HT_3 receptor have been found in the nucleus accumbens of the rat, which prompted us to determine whether dopamine would be involved in the increase of waking following microinjection of *m*-chlorophenylbiguanide into that structure (Hagan et al., 1987; Barnes et al., 1990; Chen et al., 1991). In agreement with our proposal, selective blockade of the dopamine D_1 or D_2 receptor with (+)-SCH 23390 or YM-09151-2, respectively antagonized the increase of waking and decrease of slow wave sleep after injection of *m*-chlorophenylbiguanide into the nucleus accumbens. The proposed synergistic interaction between dopamine D_1 and D_2 receptor agonists within the nucleus accumbens (Jackson et al., 1987; Dreher and Jackson, 1989; Plaznik et al., 1989) is consistent with our findings showing that dopamine D_1 or D_2 receptor antagonists prevent the action of the 5-HT_3 receptor agonist on sleep and waking.

It is worth mentioning that either *m*-chlorophenylbiguanide or YM-09151-2 alone had no effect on REM sleep variables. In contrast, the combination of the 5-HT_3 receptor agonist plus the dopamine D_2 receptor

antagonist significantly suppressed REM sleep time and the number of REM sleep periods.

The importance of the cholinergic system in the generation of REM sleep is well documented, and the mediodorsal pontine tegmentum (nuclei LCα and peri LCα) may represent a cholinoceptive REM sleep generator (Vanni-Mercier et al., 1989; Velazquez-Moctezuma et al., 1991). Although the effect of *m*-chlorophenylbiguanide on the REM sleep generator has not been established, in vitro and in vivo studies have shown that selective activation of 5-HT_3 receptors inhibit the release of acetylcholine in the cortex (Barnes et al., 1989).

In relation to the dopamine D_2 receptor antagonist YM-09151-2, a dose larger than the one given in the present study (1.0 mg/kg) significantly reduced REM sleep duration and increased REM sleep latency in the rat (Monti et al., 1989). Thus, it could be tentatively suggested that a decrease of acetylcholine release dependent on the activation of 5-HT_3 receptors, added to a blockade of dopamine D_2 receptors would be responsible for the suppression of REM sleep. However, further studies are needed to clarify the functional relationship between the nucleus accumbens neurotransmitter systems and the cholinoceptive REM sleep generator located in the pontine tegmentum.

In conclussion, the 5-HT_3 receptor agonist *m*-chlorophenylbiguanide injected into the nucleus accumbens shows the ability to increase waking and reduce slow wave sleep in rats. These effects are prevented by pretreatment with the 5-HT_3 receptor antagonist MDL 72222, the dopamine D_1 receptor antagonist (+)-SCH 23390 and the dopamine D_2 receptor antagonist YM-09151-2.

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