ELSEVIER

European Journal of Pharmacology 364 (1999) 89–98

ejp

Effects of accumbens *m*-chlorophenylbiguanide microinjections on sleep and waking in intact and 6-hydroxydopamine-treated rats

Jaime M. Monti [a,*], Ana Ponzoni [a], Héctor Jantos [a], Patricia Lagos [b], Rodolfo Silveira [b], Patricia Banchero [a]

[a] *Department of Pharmacology and Therapeutics, School of Medicine, Clinics Hospital, Zudañez 2833 / 602, Montevideo 11600, Uruguay*
[b] *Division of Cellular Biology, Institute of Biological Sciences 'Clemente Estable', Montevideo 11600, Uruguay*

Received 30 June 1998; revised 30 October 1998; accepted 6 November 1998

Abstract

Effects of the 5-HT_3 receptor agonist, *m*-chlorophenylbiguanide (10.0–40.0 μg), on sleep and waking were studied in control, vehicle-treated and 6-hydroxydopamine-injected rats. Bilateral injections of *m*-chlorophenylbiguanide into the nucleus accumbens of the control and the vehicle-infused animals significantly increased waking and reduced slow wave sleep. Rapid eye movement sleep (REM sleep) remained unchanged. Pretreatment with the selective 5-HT_3 receptor antagonist, MDL 72222 (1a*H*,3a,5a,*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 in the control group. Administration of the 5-HT_3 receptor agonist to the 6-hydroxydopamine-treated animals modified only slightly the time spent in wakefulness and slow wave sleep, while REM sleep was significantly and dose dependently reduced. Our findings further support the proposal that increase of wakefulness and reduction of slow wave sleep after activation of 5-HT_3 receptors, is partly related to the release of endogenous dopamine.

Keywords: Sleep; Waking; REM (rapid eye movement) sleep; *m*-Chlorophenylbiguanide; 5-HT_3 receptor; Nucleus accumbens; Dopamine

1. Introduction

Specific 5-HT_3 receptor binding sites have been found in the nucleus accumbens, striatum and substantia nigra (Kilpatrick et al., 1987; Waeber et al., 1990; Gehlert et al., 1991; Laporte et al., 1992a). The presence in these areas of neurons containing serotonin 5-HT_3 receptors has been confirmed by in situ hybridization (Tecott et al., 1993) and immunocytochemical analysis (Morales et al., 1996). The presynaptic location of 5-HT_3 receptors on the terminals of dopamine-containing neurons in the rat brain also has been shown on the basis of in vitro and in vivo experiments. Thus, 5-HT_3 receptor agonists such as 2-methyl-5-HT and phenylbiguanide modulate the release of dopamine in the nucleus accumbens (Jiang et al., 1990; Chen et al., 1991), and the striatum (Blandina et al., 1989). In addition, specific labelling by serotonin 5-HT_3 receptor radioligands has been confirmed in the dorsal raphe nucleus (Laporte et al., 1992b). The serotonin 5-HT_3 receptor also modulates serotonin release in the frontal cortex, the hypothalamus and the raphe nuclei of the rat (Galzin and Langer, 1991).

Recently, we showed that bilateral injection of the 5-HT_3 receptor agonist, *m*-chlorophenylbiguanide, into the nucleus accumbens of the rat, significantly increased waking and reduced slow wave sleep. Rapid eye movement (REM) sleep showed slight but inconsistent changes. Pretreatment with the 5-HT_3 receptor antagonist, MDL 72222 (1a*H*,3a,5a,*H*-tropan-3-yl-3,5-dichloro-benzoate), antagonized the increase of waking and reduction of slow wave sleep induced by *m*-chlorophenylbiguanide (Ponzoni et al., 1995). Similar results were obtained after blockade of the dopamine D_1 or D_2 receptor with (+)-SCH 23390 [(*R*)-(+)-7-chloro-8-hydroxy-2-(di-*n*-propylamino)tetralin)] or YM-09151-2 [*cis*-*N*-(1-benzyl-2-methyl-pyrrolidin-3yl)-5-chloro-2-methoxy-4-methylaminobenzamide], respectively. When given alone, either receptor antagonist slightly reduced slow wave sleep latency, without modifying values corresponding to sleep or waking (Ponzoni et al., 1995).

* Corresponding author. Tel.: +598-2-710-58-07; Fax: +598-2-487-37-87; E-mail: jmonti@chasque.apc.org

0014-2999/99/$ - see front matter
PII: S0014-2999(98)00826-7

Our results suggest that the increase of wakefulness after injection of the 5-HT_3 receptor agonist into the nucleus accumbens could be partly related to the release of endogenous dopamine.

The present study was designed to gain further insight into the role of dopamine in the *m*-chlorophenylbiguanide-induced changes in sleep and waking in the rat. To this purpose, the 5-HT_3 receptor agonist was injected into the nucleus accumbens of animals with dopamine-depleted central regions after intracerebroventricular (i.c.v.) administration of the neurotoxin, 6-hydroxydopamine. In addition, we tested the potential use of the highly selective and potent 5-HT_3 receptor antagonist, MDL 72222, against m-chlorophenylbiguanide-induced changes in sleep variables.

2. Materials and methods

Two groups of male Wistar rats weighing 320–350 g were anesthetized with sodium pentobarbital (40.0 mg/kg, i.p.). Next, 200 μg 6-hydroxydopamine hydrobromide (Sigma, USA) dissolved in 20 μl saline–ascorbate vehicle ($n = 6$) or vehicle alone ($n = 7$) was injected into the left lateral ventricle (0.8 mm posterior to bregma; 1.4 mm lateral from the midline, and 4.0 mm below the top of the skull; coordinates according to Paxinos and Watson (1986). Immediately thereafter the animals were implanted with electrodes for chronic recording of electroencephalogram and electromyogram activities from the frontal and occipital cortex and from the dorsal neck musculature. Stainless steel cannulae (25-gauge) were positioned bilaterally so that their tips were placed 2 mm above the nucleus accumbens (2.0 mm anterior to bregma, 1.5 mm lateral from the midline, and 5.6 mm below the top of the skull; coordinates according to Paxinos and Watson (1986).

A third group of rats ($n = 6$) was used to test the effect of the 5-HT_3 receptor antagonist, MDL 72222, on the *m*-chlorophenylbiguanide-induced changes in sleep variables. To this purpose, the animals were implanted as described above with electrodes for chronic recording of electroencephalogram and electromyogram activities, and with guide cannulae for microinjection into the accumbens nuclei.

Drug or vehicle (saline) was injected into the nucleus accumbens with an injection cannula (31-gauge) extending 2 mm below the guide cannula.

The rats were housed individually in a temperature-controlled room (22 ± 1°C), under a 12-h light/12-h dark cycle (lights went on at 0700 h), and with food and water ad libitum. Seven days after surgery the animals were habituated to the cable connector for 4 days in a soundproof chamber. The electroencephalographic and electromyographic activity in 25-s epochs was analysed and assigned to the following categories: 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.

2.1. The effects of the 5-HT_3 receptor agonist were studied following three different protocols, in three different groups of animals

2.1.1. Experiment 1

We studied the effect of *m*-chlorophenylbiguanide hydrochloride (Cookson, UK) 10.0, 20.0 or 40.0 μg as base, injected bilaterally into the nucleus accumbens of animals pretreated intracerebroventricularly with saline–ascorbate vehicle. Drug or vehicle (saline) was infused in a volume of 0.5 μl over a period of 60 s and the injection cannula was left in situ for another 60 s. There were seven animals in each group, and each rat received four injections.

2.1.2. Experiment 2

In the second set of experiments, *m*-chlorophenylbiguanide (10.0–20.0 or 40.0 μg) or saline was injected into the nucleus accumbens of animals pretreated i.c.v. with 6-hydroxydopamine. Six rats were in each group. They received four injections each.

2.1.3. Experiment 3

In the third set of experiments, *m*-chlorophenylbiguanide (10.0 or 20.0 μg) was injected into animals pretreated with MDL 72222 (Merrel Dow, USA) 0.5 mg/kg, s.c. There were six animals in each experimental group, and each rat received five microinjections. MDL 72222 was dissolved in a small volume of glacial acetic

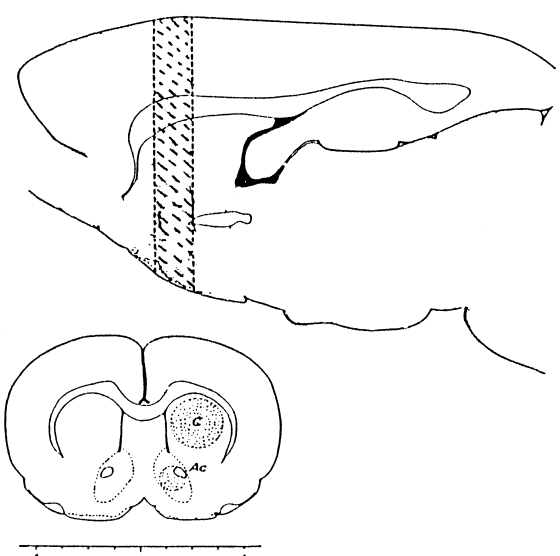

Fig. 1. Schematic illustration of the brain with indication of cut lines (——) for sampling of brain regions analyzed for dopamine and its metabolites. The sagittal section of the rat brain indicates the frontal slice (hatched bars) that was prepared for further dissection as indicated in the insert below. Tissue pieces sampled for assay of dopamine and its metabolites are shown: C = striatum; Ac = nucleus accumbens.

acid and diluted with saline; the pH was adjusted to 6.0. Subcutaneous injections were given in a final volume of 1.0 ml/kg. The drugs were given 15 min apart in the interaction experiments.

A balanced order of drug and control injections was always used to merge effects of both the drug and the time elapsed during the protocol.

Immediately after the injection into the nucleus accumbens a 6-h recording was started at approximately 0800 h. At least 4–5 days were allowed to elapse between experiments to avoid long-lasting and rebound effects on sleep.

On completion of the study (30–32 days after administration of either 6-hydroxydopamine or saline–ascorbate vehicle), the animals were killed and cannula placements

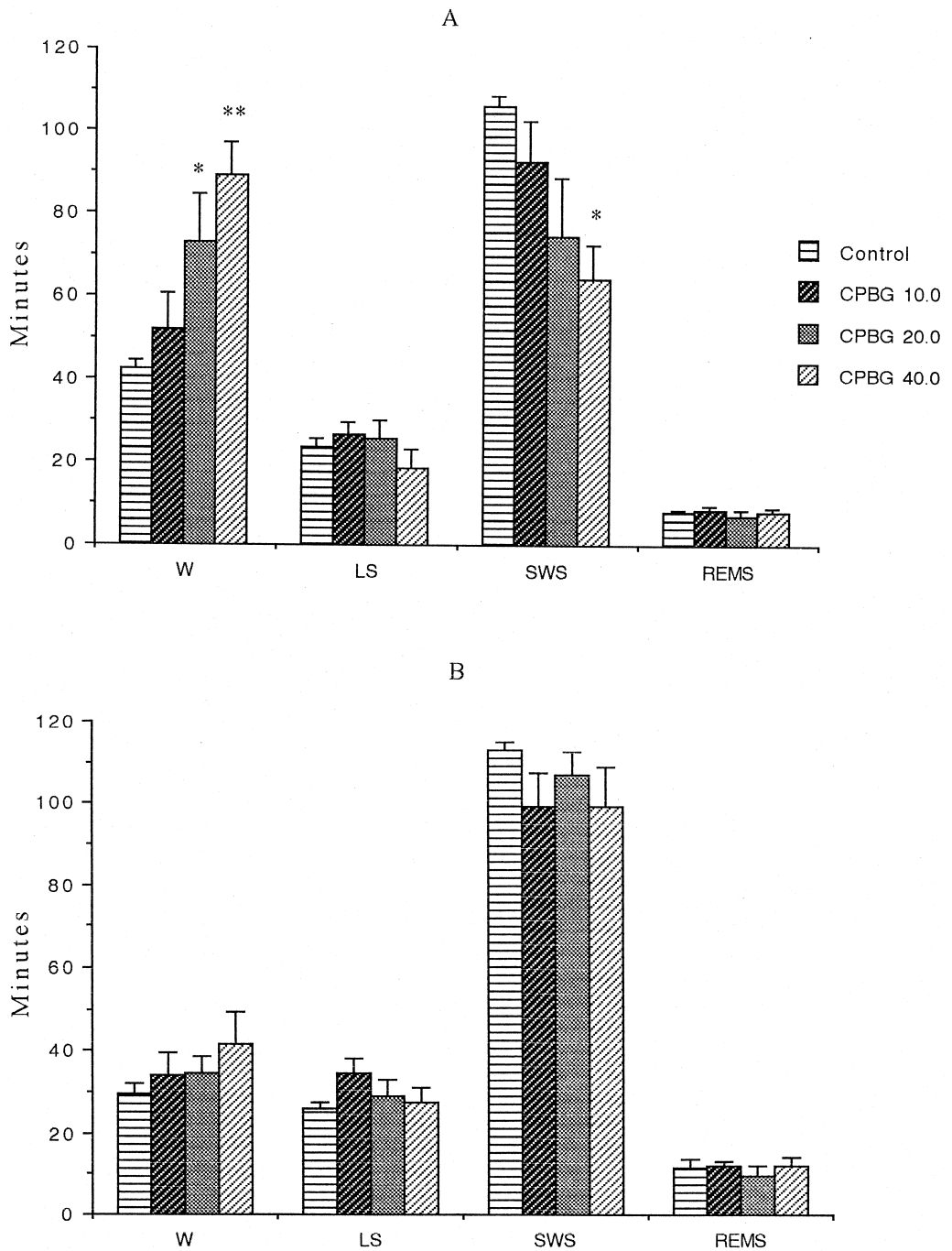

Fig. 2. The effect of *m*-chlorophenylbiguanide (CPBG) injected into the nucleus accumbens of vehicle-treated animals on wakefulness (W), light sleep (LS), slow wave sleep (SWS) and REM sleep (REMS) during successive 3-h periods (A and B). Ordinate: mean amount in min of behavioral state according to criteria described in Section 2. All values are the means (min) ± S.E.M. Seven animals were in each experimental group. Dose in μg. Compared with control values: $^{*}P < 0.02$; $^{**}P < 0.001$ (Newman–Keuls test).

Table 1
Effects of *m*-chlorophenylbiguanide injected into the accumbens nucleus on sleep latencies and number of REM periods in vehicle-treated rats

	Slow wave sleep latency (min)	REM sleep latency (min)	No. of REM periods	
			0–3 h	4–6 h
Control	12.6±4.0	100.8±28.5	2.3±0.6	5.3±1.1
m-Chlorophenylbiguanide				
10.0 μg	13.9±3.4	99.4±23.0	3.3±0.7	7.3±0.7
20.0 μg	16.0±1.8	126.3±12.3	1.4±0.6	4.9±1.4
40.0 μg	16.6±4.2	122.9±15.6	1.4±0.4	5.7±1.2

All values are the means±S.E.M.
Seven animals were in each experimental group.
No significant differences from control were observed.

were defined histologically. Correctness of cannula/injection sites was assessed using the atlas of Paxinos and Watson (1986) following a 0.5-μl injection of Fast-green dye in the accumbens nuclei. All data presented in this report are derived from animals whose injection sites were within the limits of the accumbens nuclei.

2.2. Biochemical assays

Two additional groups of rats were injected into the left lateral ventricle with either 6-hydroxydopamine (200 μg) dissolved in saline–ascorbate vehicle ($n=5$) or with vehicle alone ($n=5$), as described above. Thirty days later, the animals were killed and the brains were removed. The brains were placed in ice-cold NaCl 0.9% solution and allowed to cool for 1 min. Thereafter a frontal slice 2 mm thick was made using a device consisting of two long razor blades kept 2 mm apart. The slice was transferred to a thin metal plate and placed in a freezer at −20°C. After freezing, the striatum and the nucleus accumbens were punched using stainless steel punches ($Ø=1$ or 2 mm) (Fig. 1).

Levels of dopamine and of its metabolites [dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA)] were assayed in the left and right nucleus accumbens and striatum, using high pressure liquid chromatography with electrochemical detection (Monti et al., 1995).

2.3. Statistical analysis

One-way analysis of variance (ANOVA) with multiple measures was used in the sleep studies for comparison of four or more samples, with multiple post-hoc comparisons performed with the Newman–Keuls test when the ANOVA indicated significance. The *t*-test for unpaired samples was used in the biochemical assays for comparison of two samples.

3. Results

3.1. Effects of m-chlorophenylbiguanide in the saline–ascorbate vehicle-infused animals

Following bilateral intra-accumbens injection of *m*-chlorophenyl-biguanide (20.0 or 40.0 μg) to the vehicle-infused animals, wakefulness was significantly increased, whereas slow wave sleep was reduced. As compared to control values, light sleep, REM sleep, sleep latencies and the number of REM periods showed no significant changes. Effects of the 5-HT_3 receptor agonist on sleep variables were evident only during the first 3 h of recording (Fig. 2 and Table 1).

3.2. Effects of m-chlorophenylbiguanide in the 6-hydroxydopamine-infused animals

6-Hydroxydopamine administration induced a significant dopamine and HVA reduction in the nucleus accumbens and the striatum. Levels of DOPAC also were significantly reduced in the striatum (Fig. 3).

As compared to the vehicle-treated animals, the 6-hydroxydopamine-treated rats had waking reduced ($P<0.02$) whereas slow wave sleep was increased ($P<0.05$) during the first three recording h of the control session (Fig. 4). Administration of *m*-chlorophenylbiguanide to the 6-hydroxydopamine-treated animals modified only slightly the amount of time spent in wakefulness, light sleep or slow

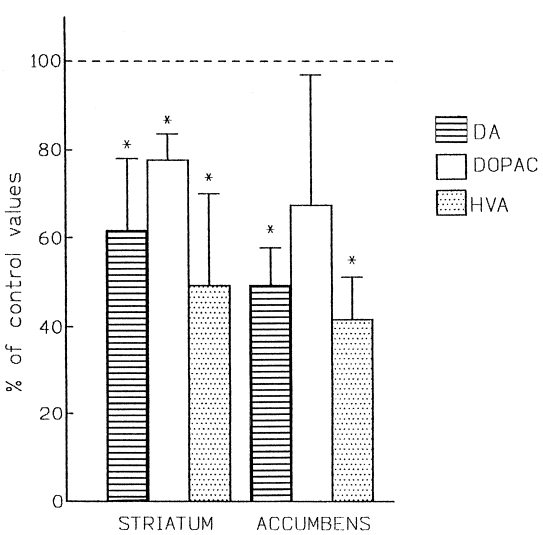

Fig. 3. Effect of intracerebroventricular 6-hydroxydopamine treatment on dopamine (DA), dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) levels in the striatum and nucleus accumbens of adult rats. Each bar represents the mean±S.E.M. of five determinations, expressed as % of respective control value ($n=5$). $^{*}P<0.05$ (*t*-test for unpaired samples). Absolute values for striatum—DA: 14500±2059 ng/g; DOPAC: 3352±279 ng/g; HVA: 1800±347 ng/g. Absolute values for nucleus accumbens—DA: 10053±1724 ng/g; DOPAC: 3681±676 ng/g; HVA: 1736±519 ng/g.

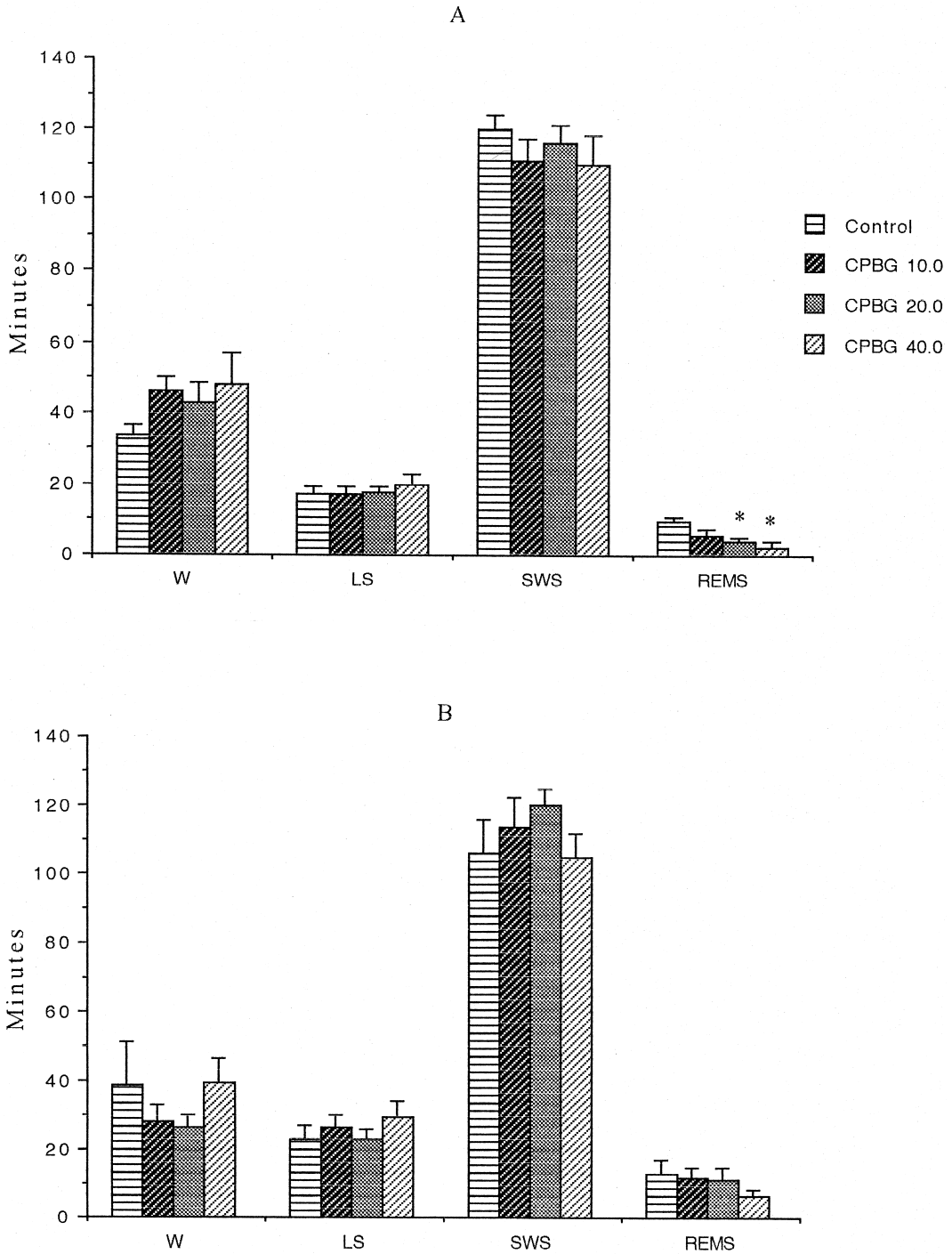

Fig. 4. The effect of *m*-chlorophenylbiguanide injected into the nucleus accumbens of 6-hydroxydopamine-treated animals on sleep and waking during successive 3-h periods (A and B). Six animals were in each experimental group. Dose in μg. Compared with control values: $^{*}P < 0.05$ (Newman–Keuls test).

wave sleep. On the other hand, REM sleep was significantly and dose dependently reduced after the 20.0- and 40.0-μg doses during the first three h after administration (Fig. 4). REM sleep latency showed a significant increase after the 40.0-μg dose, whereas the number of REM periods was decreased after the whole range of doses, during the first 3 h of recording (Table 2).

3.3. Effects of m-chlorophenylbiguanide and MDL 72222 in the control group of animals

As described for the vehicle-infused group of rats, bilateral intra-accumbens injection of *m*-chlorophenylbiguanide (10.0–40.0 μg) in a control group of animals reduced slow wave sleep and produced a significant in-

Table 2
Effects of *m*-chlorophenylbiguanide injected into the nucleus accumbens on sleep latencies and number of REM periods in 6-hydroxydopamine-treated rats

	Slow wave sleep latency (min)	REM sleep latency (min)	No. of REM periods	
			0–3 h	4–6 h
Control	15.0 ± 2.1	93.7 ± 16.6	6.2 ± 0.7	6.2 ± 1.6
m-Chlorophenylbiguanide				
10.0 μg	21.2 ± 4.8	97.3 ± 20.4	3.5 ± 1.1[a]	7.7 ± 1.0
20.0 μg	12.5 ± 2.9	95.0 ± 19.0	2.5 ± 1.0[a]	5.2 ± 1.6
40.0 μg	13.0 ± 5.3	175.7 ± 43.4[b]	1.5 ± 0.8[b]	4.0 ± 0.8

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

crease in wakefulness, whereas REM sleep remained unchanged (Fig. 5). Pretreatment with MDL 72222 (0.5 mg/kg) prevented the *m*-chlorophenylbiguanide-induced changes in sleep variables (Fig. 6).

4. Discussion

2-Methyl-5-HT, phenylbiguanide and *m*-chlorophenylbiguanide are the preferred ligands with which to activate serotonin 5-HT_3 receptors selectively. *m*-Chlorophenylbiguanide is appreciably more potent than either 2-methyl-5-HT or phenylbiguanide and, unlike the latter, has no significant effects at other 5-HT receptor sites (Fozard, 1990; Kilpatrick et al., 1990; Zifa and Fillion, 1992).

In the present study, *m*-chlorophenylbiguanide 10.0, 20.0 and 40.0 μg was infused bilaterally in a volume of 0.5 μl. It could be argued that 40.0 μg *m*-chlorophenylbiguanide is too large a dose to be injected into the nucleus accumbens, and that the latter was not the only region involved in the effects observed. However, following the 20.0-μg dose sleep variables also were significantly modified, which tends to favour the possibility of a selective effect on the nucleus accumbens.

Acute injection of *m*-chlorophenylbiguanide into the nucleus accumbens of control and vehicle-infused animals increased wakefulness and reduced slow wave sleep without suppressing REM sleep. The 5-HT_3 receptor antagonist, MDL 72222, prevented the reduction of slow wave sleep and increase of wakefulness in the control group of rats. These results are in agreement with those of a previous study from our laboratory, which showed that the 5-HT_3 receptor agonist significantly decreased slow wave sleep and slightly reduced REM sleep (Ponzoni et al., 1995).

The 6-hydroxydopamine treatment used in this study depleted nucleus accumbens and striatum dopamine by 50.2% and 38.0%, respectively. It could be disputed that our experimental approach was not the most appropriate, because 6-hydroxydopamine given i.c.v. induced a depletion of both dopamine and noradrenaline at central sites. However, it should be taken into consideration that 6-hydroxydopamine lesions performed bilaterally at several sites along the dopamine-containing pathway in rats induce a significant depletion of dopamine but also severe adipsia and aphagia, hypoactivity, difficulties with initiation of activity and loss of exploratory behavior. As a result the animals die within 5–8 days (Ungerstedt, 1971). On the other hand, i.c.v. administration of 6-hydroxydopamine either does not alter locomotor activity (Burkard et al., 1969), or induces a reversible reduction of spontaneous motor behavior (Evetts et al., 1970). The differences in effects could depend on 6-hydroxydopamine partly sparing the nigro-striatal pathway, while effectively depleting dopamine at mesolimbic and mesocortical sites.

Slow wave sleep was significantly increased and wakefulness reduced in the 6-hydroxydopamine-treated group, as compared to the vehicle-infused rats. On the other hand, REM sleep was not significantly modified. Pharmacological studies, particularly those with selective noradrenaline (α_1) or dopamine (D_1 and D_2) receptor agonists, have revealed that both neurotransmitter systems play a role in the regulation of the waking state (Monti, 1982; Monti et al., 1988; Wauquier, 1995). Thus, as pointed out by Lidbrink and Fuxe (1973), catecholamine depletion after treatment with 6-hydroxydopamine could have been responsible for the reduction of waking and increase of slow wave sleep.

Intra-accumbens administration of *m*-chlorophenylbiguanide to the 6-hydroxydopamine-injected group of animals only slightly modified wakefulness and slow wave sleep. Nevertheless, REM sleep and the number of REM periods showed a statistically significant reduction during the first 3 h of recording, while REM sleep latency was increased.

Our findings in animals with significant depletion of dopamine in the nucleus accumbens further support the proposal that the increase of wakefulness and reduction of slow wave sleep after selective activation of the serotonin 5-HT_3 receptor is partly related to the release of endogenous dopamine. Moreover, the concomitant activation of both dopamine D_1 and D_2 receptor-mediated mechanisms in the nucleus accumbens seems to be essential for increasing wakefulness (Ponzoni et al., 1995). The assumption of specificity of *m*-chlorophenylbiguanide is supported by the finding that MDL 72222 effectively prevented its effects.

As mentioned above, REM sleep values were not significantly different in the 6-hydroxydopamine-treated group and in the vehicle-injected rats. In this respect, the proposal that identifies cholinergic neurons in the laterodorsal and pedunculopontine tegmental nuclei with REM sleep promotion, and the inhibition of these neurons by serotonergic afferents from the dorsal raphe nuclei and noradrenergic afferents from the locus coeruleus, is the one that best fits the experimental evidence to elucidate REM sleep (McCarley, 1983). Current electrophysiological and phar-

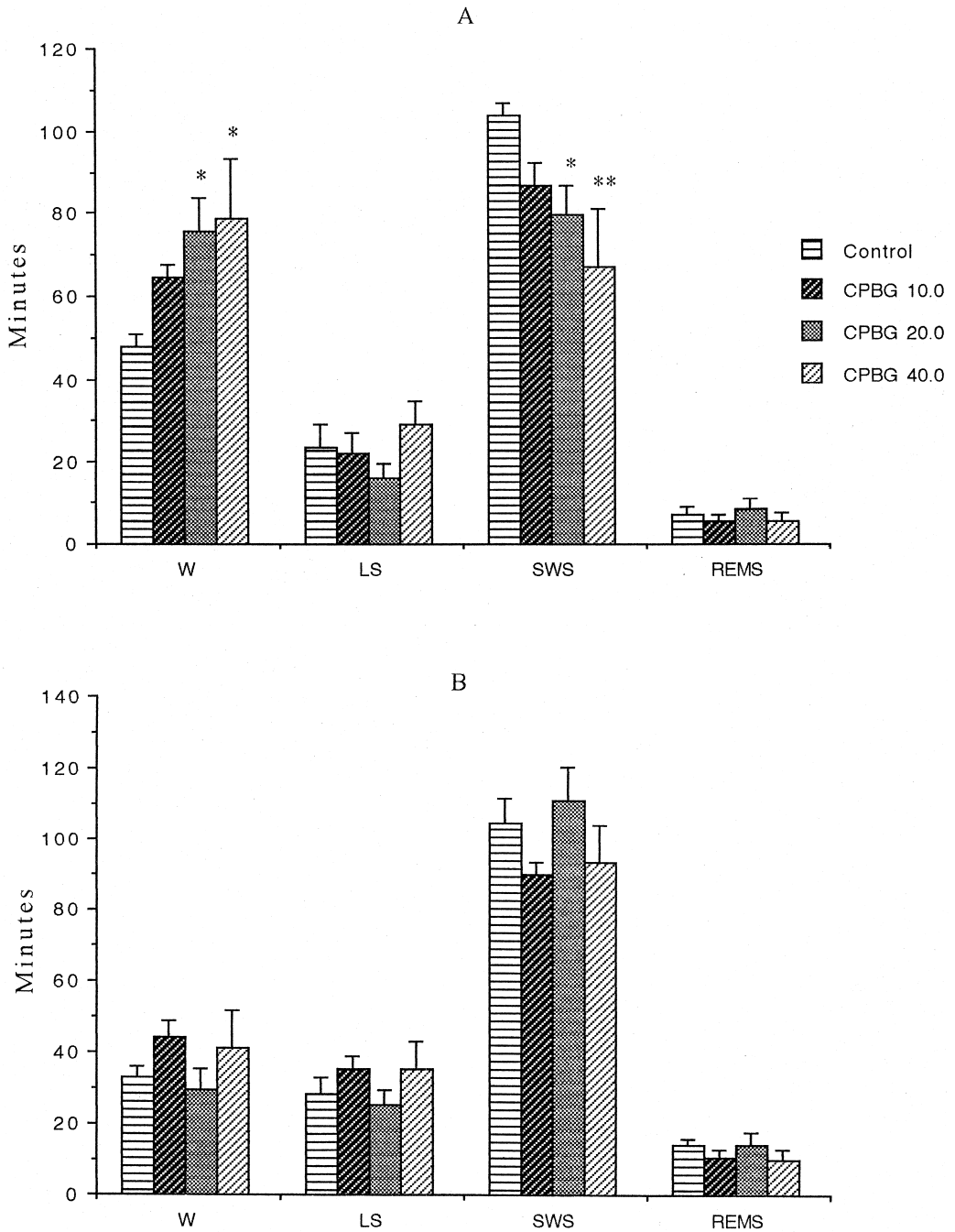

Fig. 5. The effect of *m*-chlorophenylbiguanide injected into the nucleus accumbens of control animals on sleep and waking during successive 3-h periods (A and B). Six animals were in each experimental group. Dose in μg. Compared with control values: $^{*}P < 0.03$; $^{**}P < 0.01$ (Newman–Keuls test).

macological data strongly support the hypothesis that noradrenaline can inhibit REM sleep (McCarley et al., 1995). In agreement with this proposal, Hartmann et al. (1971) found that REM sleep was significantly increased 2–3 weeks after administration of 6-hydroxydopamine (500 μg) to rats. However, this observation could not be confirmed by Matsuyama et al. (1973), since the REM sleep values for their 6-hydroxydopamine-treated animals were similar to those found in the vehicle-treated group. Moreover, in the study by Lidbrink and Fuxe (1973) where 6-hydroxydopamine (2–16 μg) was injected in the mesencephalon of the rat, causing small and selective lesions of the dorsal noradrenaline pathway, REM sleep was not significantly different from that of the control group. Thus, following administration of 6-hydroxydopamine, both an increase of REM sleep and the absence of any clear effect have been described in the rat. Hartmann et al. (1971) used young rats, whereas we and Matsuyama et al. (1973) used adult rats. According to Zigmond and Keefe (1997) the behavioral and neurobiological consequences of injections

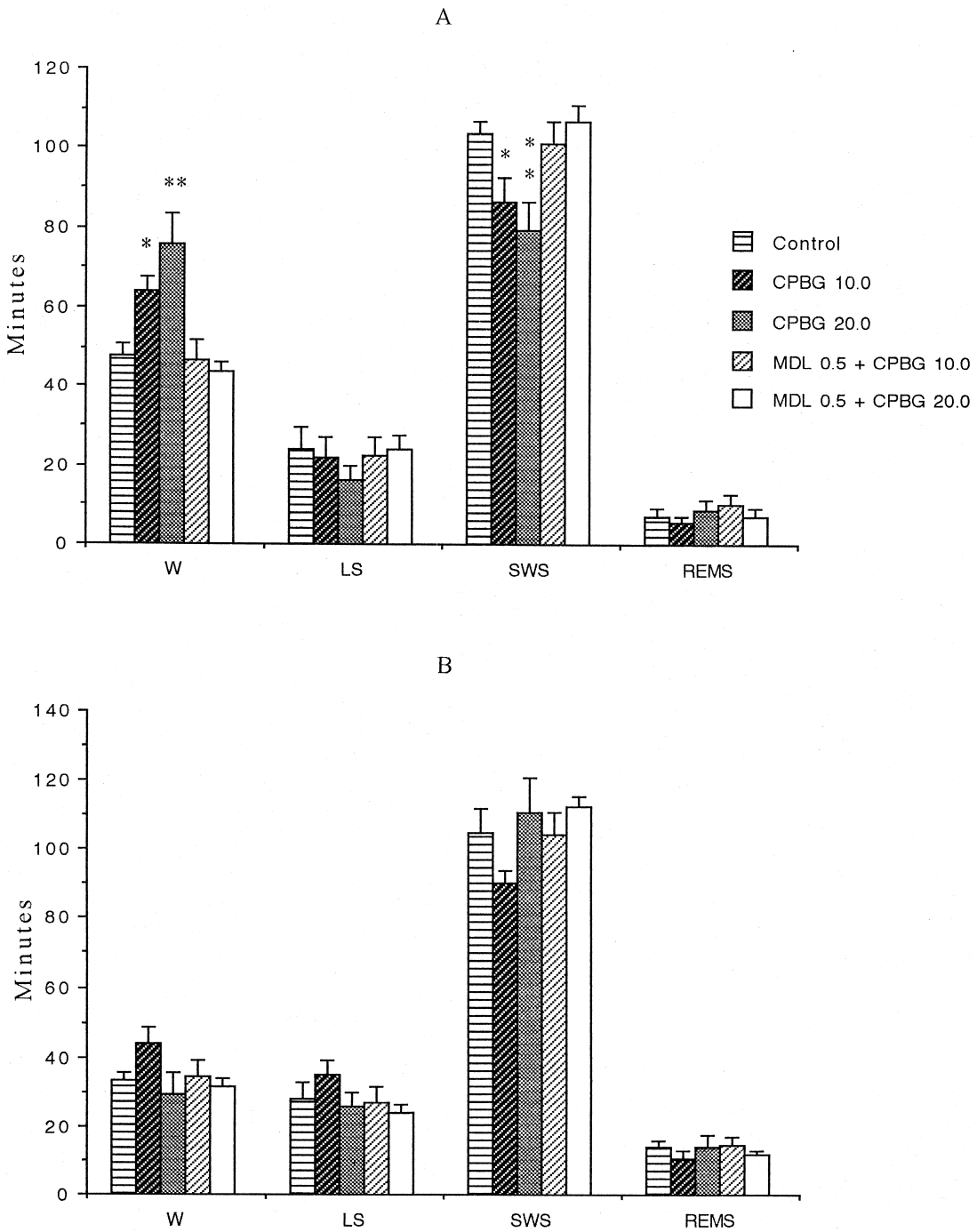

Fig. 6. The effect of pretreatment with MDL 72222 on the *m*-chlorophenylbiguanide-induced changes in sleep and waking during successive 3-h periods (A and B). Six animals were in each group. Dose in μg. Compared with control values: $^{*}P < 0.02$; $^{**}P < 0.01$ (Newman–Keuls test).

of 6-hydroxydopamine into young rats can be quite different from those of injections into adult animals. Thus, the differences in REM sleep values after 6-hydroxydopamine injection could had been partly related to the age of the rats. Rat strain or sex does not seem to have been explored with respect to 6-hydroxydopamine effects.

Recently, we showed that i.c.v. administration of *m*-chlorophenylbiguanide to intact rats suppresses REM sleep (Ponzoni et al., 1993). In contrast, REM sleep remains unchanged after direct injection of the 5-HT_3 receptor agonist into the nucleus accumbens of control animals (Ponzoni et al., 1995). On the other hand, nucleus accumbens administration of *m*-chlorophenylbiguanide to 6-hydroxydopamine-treated animals induced a decrease of REM sleep.

It has been reported that serotonin 5-HT_3 receptor stimulation with 2-methyl-5-HT facilitates 5-HT release from the somatodendritic area of serotonergic neurons in

the raphe nuclei, and from axon terminals in the hypothalamus and the hippocampus of the rat (Blier and Bouchard, 1993; Bagdy et al., 1998).

Moreover, the dorsal raphe nucleus contains a fairly high density of dopamine D_2-receptors but not dopamine D_1 receptors (Bouthenet et al., 1987). Stimulation of dopamine D_2 receptors in the dorsal raphe nucleus with the dopamine D_2/D_3 receptor agonist, quinpirole, increases serotonin levels, which is prevented by the specific dopamine D_2 receptor antagonist, raclopride (Ferré and Artigas, 1993).

On the basis of these findings it could be suggested that: (a) REM sleep suppression in animals treated with *m*-chlorophenylbiguanide i.c.v. is dependent on the release of dopamine from the nucleus accumbens and the striatum (Blier and Bouchard, 1993), and of serotonin from axon terminals in the pontine reticular formation where cholinergic REM sleep promoting neurons are located (Sanford et al., 1994); (b) dopamine released from the nucleus accumbens after local injection of *m*-chlorophenyl-biguanide in control or vehicle-treated animals could have increased serotonin levels to an extent insufficient to suppress REM sleep; (c) in animals with depletion of dopamine at central sites and denervation hypersensitivity of dopamine D_2 receptors in the dorsal raphe, the serotonin 5-HT_3 receptor agonist could have induced a much greater response, and consequently suppression of REM sleep. In other words, 6-hydroxydopamine could have amplified the effect of *m*-chlorophenyl-biguanide microinjection on this behavioral state. Further studies are needed to resolve this issue.

Acknowledgements

The authors are grateful to Dr. Miguel Reyes-Parada for the critical discussions during the preparation of this manuscript and to Ms. Nilda Acuña for her valuable technical assistance. This work was partially supported by Conicyt grants 01/94 and 038/94.

References

Bagdy, E., Solyom, S., Harsing, L.G., 1998. Feedback stimulation of somatodendritic serotonin release: a 5-HT_3 receptor-mediated effect in the raphe nuclei of the rat. Brain Res. Bull. 45, 203–208.

Blandina, P., Goldfarb, J., Craddock-Royal, B., Green, J.P., 1989. Release of endogenous dopamine by stimulation of 5-hydroxytryptamine$_3$ receptors in rat striatum. J. Pharmacol. Exp. Ther. 251, 803–809.

Blier, P., Bouchard, C., 1993. Functional characterization of a 5-HT_3 receptor which modulates the release of 5-HT in the guinea-pig brain. Br. J. Pharmacol. 108, 13–22.

Bouthenet, M.L., Martres, M.P., Sales, N., Schwartz, J.C., 1987. A detailed mapping of dopamine D-2 receptors in rat central nervous system by autoradiography with [^{125}I]iodosulpiride. Neuroscience 20, 117–155.

Burkard, W.P., Jalfre, M., Blum, J., 1969. Effect of 6-hydroxydopamine on behavior and cerebral amine content in rats. Experientia 25, 1295–1296.

Chen, J., Van Praag, H.M., Gardner, E.L., 1991. Activation of 5-HT_3 receptor by 1-phenylbiguanide increases dopamine release in the rat nucleus accumbens. Brain Res. 543, 353–357.

Eveits, K.D., Uretsky, N.J., Iversen, L.L., Iversen, S.D., 1970. Effects of 6-hydroxydopamine on CNS catecholamines, spontaneous motor activity and amphetamine induced hyperactivity in rats. Nature 225, 961–962.

Ferré, S., Artigas, F., 1993. Dopamine D_2 receptor-mediated regulation of serotonin extracellular concentration in the dorsal raphe nucleus of freely moving rats. J. Neurochem. 61, 772–775.

Fozard, J.R., 1990. Agonists and antagonists of 5-HT_3 receptors. In: Saxena, P.R., Wallis, D.I., Wouters, W., Bevan, P. (Eds.), Cardiovascular Pharmacology of 5-Hydroxytryptamine. Kluwer, Dordrecht, pp. 101–115.

Galzin, A.M., Langer, S.Z., 1991. Modulation of 5-HT release by presynaptic inhibitory and facilitatory 5-HT receptors in brain slices. In: Langer, S.Z., Galzin, A.M., Costentin, J. (Eds.), Presynaptic Receptors and Neuronal Transporters. Adv. Biosci. 82, 59–62.

Gehlert, D.R., Gackenheimer, S.L., Wong, D.T., Robertson, D.W., 1991. Localization of 5-HT_3 receptors in the rat brain using [^{3}H]LY278584. Brain Res. 553, 149–154.

Hartmann, E., Chung, R., Draskoczy, P.R., Schildkraut, J.J., 1971. 6-Hydroxydopamine: effects on sleep in the rat. Nature 233, 425–427.

Jiang, L.H., Ashby, C.R., Kasser, R.J., Wang, R.Y., 1990. The effect of intra-ventricular administration of the 5-HT_3 receptor agonist 2-methylserotonin on the release of dopamine in the nucleus accumbens: an in vivo chronocoulometric study. Brain Res. 513, 156–160.

Kilpatrick, G.J., Jones, B.J., Tyers, M.B., 1987. Identification and distribution of 5-HT_3 receptors in rat brain using radioligand binding. Nature 330, 746–748.

Kilpatrick, G.J., Bunce, K.T., Tyers, M.B., 1990. 5-HT_3 receptors. Med. Res. Rev. 10, 441–475.

Laporte, A.M., Koscielniak, T., Ponchant, M., Verge, D., Hamon, M., Golzan, H., 1992a. Quantitative autoradiographic mapping of 5-HT_3 receptors in the rat CNS using [^{125}I]iodozacopride and [^{3}H]zacopride as radioligands. Synapse 10, 271–281.

Laporte, A.M., Kidd, E.J., Vergé, D., Gozlan, H., Hamon, M., 1992b. Autoradiographic mapping of central 5-HT_3 receptors. In: Hamon, M. (Ed.), Central and Peripheral 5-HT_3 Receptors. Academic Press, London, pp. 157–187.

Lidbrink, P., Fuxe, K., 1973. Effects of intracerebral injections of 6-hydroxydopamine on sleep and waking in the rat. J. Pharm. Pharmacol. 25, 84–87.

Matsuyama, S., Coindet, J., Mouret, J., 1973. 6-Hydroxydopamine intracisternale et sommeil chez le rat. Brain Res. 57, 85–95.

McCarley, R.W., 1983. REM dreams, REM sleep and their isomorphisms, In: Chase, M., Weitzman, E.D. (Eds.), Sleep Disorders: Basic and Clinical Research. Spectrum, New York, pp. 363–392.

McCarley, R.W., Greene, R.W., Rainnie, D., Portas, Ch.M., 1995. Brainstem neuromodulation and REM sleep. Semin. Neurosci. 7, 341–354.

Monti, J.M., 1982. Catecholamines and the sleep–wake cycle: I. EEG and behavioral arousal. Life Sci. 30, 1145–1157.

Monti, J.M., Hawkins, M., Jantos, H., D'Angelo, L., Fernández, M., 1988. Biphasic effects of dopamine D-2 receptor agonists on sleep and wakefulness in the rat. Psychopharmacology 95, 395–400.

Monti, J.M., Jantos, H., Silveira, R., Reyes-Parada, M., Scorza, C., 1995. Sleep and waking in 5,7-DHT-lesioned or (−)-pindolol-pretreated rats after administration of buspirone, ipsapirone, or gepirone. Pharmacol. Biochem. Behav. 52, 305–312.

Morales, M., Battenberg, E., de Lecea, L., Sanna, P.P., Bloom, F.E., 1996. Cellular and subcellular immunolocalization of the type 3 serotonin receptor in the rat central nervous system. Mol. Brain Res. 36, 251–260.

Paxinos, G., Watson, C., 1986. The Rat Brain. Academic Press, Sydney.

Ponzoni, A., Monti, J.M., Jantos, H., 1993. The effects of selective

activation of the 5-HT_3 receptor with *m*-chlorophenylbiguanide on sleep and wakefulness in the rat. Eur. J. Pharmacol. 249, 259–264.

Ponzoni, A., Monti, J.M., Jantos, H., Altier, H., Monti, D., 1995. Increased waking after intra-accumbens injection of *m*-chlorophenylbiguanide: prevention with serotonin or dopamine receptor antagonists. Eur. J. Pharmacol. 278, 111–115.

Sanford, L.D., Ross, R.J., Seggos, A.E., Morrison, A.R., Ball, W.A., Mann, G.L., 1994. Central administration of two 5-HT receptor agonists: effect on REM sleep initiation and PGO waves. Pharmacol. Biochem. Behav. 49, 93–100.

Tecott, L.H., Maricq, A.V., Julius, D., 1993. Nervous system distribution of the serotonin 5-HT_3 receptor mRNA. Proc. Natl. Acad. Sci. USA 90, 1430–1434.

Ungerstedt, U., 1971. Adipsia and aphagia after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system. Acta Physiol. Scand. Suppl. 367, 95–122.

Waeber, C., Pinkus, L.M., Palacios, J.M., 1990. The (*S*)-isomer of [^{3}H]zacopride labels 5-HT_3 receptors with high affinity in rat brain. Eur. J. Pharmacol. 181, 283–287.

Wauquier, A., 1995. Pharmacology of the catecholaminergic system. In: Kales, A. (Ed.). The Pharmacology of Sleep. Springer Verlag, Berlin, pp. 65–90.

Zifa, E., Fillion, F., 1992. 5-Hydroxytryptamine receptors. Pharmacol. Rev. 44, 401–458.

Zigmond, M.J., Keefe, K.A., 1997. 6-Hydroxydopamine as a tool for studying catecholamines in adult animals. In: Kostrzewa, R.M. (Ed.), Highly Selective Neurotoxins: Basic and Clinical Applications. Humana Press, Totowa, pp. 75–107.