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Sleep and Waking in 5,7-DHT-Lesioned or (−)-Pindolol-Pretreated Rats After Administration of Buspirone, Ipsapirone, or Gepirone

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MONTI, J. M., H. JANTOS, R. SILVEIRA, M. REYES-PARADA AND C. SCORZA. *Sleep and waking in 5,7-DHT-lesioned or (−)-pindolol-pretreated rats after administration of buspirone, ipsapirone, or gepirone.* PHARMACOL BIOCHEM BEHAV **52**(2) 305-312, 1995.—The effects of partial 5-HT_{1A} receptor agonists buspirone (0.010-4.0 mg/kg), ipsapirone (0.010-6.0 mg/kg), and gepirone (0.025-4.0 mg/kg) on sleep and waking were studied in vehicle-treated and 5,7-dihydroxytryptamine (5,7-DHT)-injected rats. 5,7-DHT-treated animals showed a marked and significant serotonin and 5-HIAA depletion in the raphe regions of the pons and upper brain stem, cerebral cortex, hippocampus, and striatum. Subcutaneous administration of the partial agonists to both the vehicle-infused and the 5,7-DHT-treated animals significantly increased waking (W) and reduced light sleep (LS), slow-wave sleep (SWS), and REM sleep (REMS). Pretreatment with (−)-pindolol (2.0 mg/kg) reversed the effects of buspirone and gepirone on W and non-REM sleep (LS + SWS) whereas REMS remained suppressed. (−)-Pindolol failed to reverse the effects of ipsapirone on sleep and W. The present results tend to indicate that increased W after acute administration of buspirone, ipsapirone, or gepirone depends upon the activation of postsynaptic 5-HT_{1A} receptors. The well-known anxiolytic action observed after chronic administration of the azapirones seems to be related to mechanisms other that these involved in their stimulant effect.

Sleep Waking Buspirone Ipsapirone Gepirone Azapirones (−)-Pindolol Rat

ON THE basis of operational, structural, and transductional criteria, a new nomenclature for 5-HT receptors has been proposed (16). In this respect, the 5-HT_1 receptor subtype was subdivided into 5 different subsets that differ in their biochemical and pharmacological characteristics, and have been termed 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, and 5-HT_{1F}. The 5-HT_{1C} receptor, widely accepted as a 5-HT_2 receptor subtype, is now referred to as the 5-HT_{2C} site.

The 5-HT_{1A} receptor is found in structures belonging to the limbic system, including the hippocampus, septal nuclei, amygdala, and enthorhinal cortex, and in the dorsal and median raphe (37). 5-HT_{1A} receptors in the raphe nuclei act as somatodendritic autoreceptors regulating the firing of serotonergic neurons. On the other hand, 5-HT_{1A} receptors located in limbic structures function as postsynaptic receptors. Selective serotonergic lesions using 5,7-dihydroxytryptamine (5,7-DHT) induce a marked decrease of autoreceptor density while postsynaptic sites remain intact (36). A number of selective agonists for the 5-HT_{1A} receptor are available. They include 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT), which

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acts as full agonist at somatodendritic and postsynaptic sites, and the azapirones buspirone, ipsapirone, and gepirone, which function as full agonists at 5-HT_{1A} autoreceptors, and, depending on the paradigm used, either show partial agonist activity or virtually full antagonistic activity at postsynaptic 5-HT_{1A} receptors (8,9). The affinity values of buspirone, ipsapirone, and gepirone for the 5-HT_{1A} receptor (K_i in nM) do not differ considerably, amounting to 15, 5.5, and 26 nM, respectively (25). The K_is of buspirone, ipsapirone, and gepirone at D_2 receptor are 42, 420 and 560 nM, respectively (25), which tends to support the proposal that buspirone, through a presynaptic antagonist action on dopaminergic neurotransmission, might impact several behavioral paradigms (33).

The β-adrenoceptor antagonist pindolol, which blocks both pre- and postsynaptic 5-HT_{1A} and 5-HT_{1B} receptors, has been shown to antagonize the 8-OH-DPAT-induced behavioral syndrome in the rat (17,30). An enantiomeric selectivity has been noted, with the most potent compound associated with the (−) sign of optical rotation (14,15). Moreover, results of binding studies have shown that affinity values of (−)-pindolol for 5-HT_{1A} and 5-HT_{1B} receptor subtypes (pK_D values) are almost similar, amounting to 7.71 and 7.75, respectively (13).

Studies aimed at determining the role of 5-HT_{1A} receptor on sleep and waking (W) have shown 8-OH-DPAT to induce biphasic effects such that a low dose (0.010 mg/kg) reduced W and increased slow wave sleep (SWS) whereas a higher dose (0.375 mg/kg) induced opposite effects. REM sleep (REMS) was decreased over the whole range of doses given (22,23). Intraventricular administration of the neurotoxin 5,7-DHT resulted in lasting damage of presynaptic 5-HT structures and in the suppression of the low-dose 8-OH-DPAT-induced increase of SWS and reduction of W. On the other hand, the large-dose 8-OH-DPAT-induced increase of W and decrease of SWS was still present, thus suggesting their dependence on postsynaptic 5-HT_{1A} receptors (24).

Concerning the azapirones, buspirone (3.0–10.0 mg/kg) has been shown to increase total wake time and to decrease non-REM sleep and REMS in rats (18). In a preliminary study, ipsapirone (1.0–3.0 mg/kg) was shown to increase W and to reduce REMS. The effects of ipsapirone persisted after infusion of the neurotoxin 5,7-DHT into the dorsal raphe nucleus, thus suggesting the involvement of postsynaptic 5-HT_{1A} receptors (35).

The present study was designed to gain further insight into the mechanisms involved in the action of the azapirones buspirone, ipsapirone, and gepirone on sleep and W. To this purpose, the compounds were given over a wide range of dosages to rats pretreated with (−)-pindolol or selectively depleted of serotonin from central regions after administration of the neurotoxin 5,7-DHT.

METHOD

Animals

Male Wistar rats (School of Medicine Breeding Laboratories, Montevideo, Uruguay) weighing 320–350 g were implanted under general anesthesia (sodium pentobarbital 40.0 mg/kg) with Nichrome electrodes (200 μm diameter) for chronic sleep recording from frontal and occipital cortex and from dorsal neck musculature. Rats were injected prior to surgery with 25.0 mg/kg desipramine-HCl dissolved in distilled water, IP. Thirty minutes later, 200 μg 5,7-DHT (Sigma, USA) dissolved in 25 μl saline-ascorbate vehicle (n = 6) or vehicle alone (n = 8) 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 (26)]. The animals were housed individually in a temperature-controlled room (20 ± 1°C), under 12L : 12D cycle (lights went on at 0700 h and went off at 1900 h), and food and water were available ad lib. Ten days after surgery the animals were habituated to a sound-proof chamber fitted with slip-rings and cable connectors. The electrographic activity in 50-s periods was analysed and assigned to the following categories based on the waveform: W [characterized by low-voltage fast waves in frontal cortex, and mixed theta rhythm (4–7 Hz) in occipital cortex and relatively high EMG activity]; LS (high-voltage slow cortical waves interrupted by low-voltage fast EEG activity); SWS (continuous high-amplitude slow frontal and occipital waves combined with a reduced EMG), and REMS (low-voltage fast frontal waves, a regular theta rhythm in the occipital cortex, and a silent EMG except for occasional twitchings) (21).

Pharmacological Procedure

We studied the effect of control solution, buspirone (Bristol-Myers, USA) 0.010–4.0 mg/kg, ipsapirone (Bayer-Tro-

TABLE 1

EFFECTS OF BUSPIRONE ON WAKEFULNESS, LIGHT SLEEP, SLOW-WAVE SLEEP, AND REM SLEEP IN VEHICLE-TREATED RATS DURING 6-h SESSIONS

	0–2 h	3–4 h	5–6 h
Wakefulness			
Control	37.6 ± 5.6	24.0 ± 2.2	26.0 ± 3.2
Buspirone (mg/kg)			
0.010	39,4 ± 5.4	21.2 ± 3.8	27.6 ± 3.4
0.025	40.0 ± 3.8	24.2 ± 4.2	24.2 ± 2.4
2.0	98.6 ± 3.8*	26.6 ± 4.0	26.0 ± 3.2
4.0	104.6 ± 6.4*	44.6 ± 8.6	19.8 ± 2.6
Light sleep			
Control	20.4 ± 1.0	17.6 ± 1.4	18.0 ± 2.0
Buspirone (mg/kg)			
0.010	20.0 ± 1.6	18.6 ± 1.6	21.0 ± 2.4
0.025	17.2 ± 1.8	17.6 ± 1.4	19.6 ± 2.4
2.0	9.2 ± 2.8†	13.0 ± 2.4	18.2 ± 1.8
4.0	10.0 ± 4.2†	17.4 ± 4.0	14.0 ± 1.6
Slow-wave sleep			
Control	57.4 ± 4.8	67.0 ± 2.6	65.0 ± 3.8
Buspirone (mg/kg)			
0.010	55.0 ± 5.4	69.2 ± 2.4	57.4 ± 3.6
0.025	58.0 ± 4.4	68.2 ± 3.8	64.0 ± 3.2
2.0	12.2 ± 2.6*	71.6 ± 3.2	64.2 ± 4.8
4.0	4.8 ± 2.4*	82.0 ± 4.8	75.8 ± 3.2
REM sleep			
Control	4.6 ± 1.0	11.4 ± 2.0	11.0 ± 1.6
Buspirone			
0.010	5.6 ± 1.2	9.6 ± 0.8	14.0 ± 1.2
0.025	4.8 ± 1.0	13.4 ± 2.0	12.2 ± 1.4
2.0	0†	4.0 ± 1.4	11.6 ± 1.4
4.0	0.6 ± 0.4†	0.4 ± 0.2*	10.4 ± 1.1

All values are mean ± SEM in minutes. Eight animals were in each experimental group. *†Compared with control values: *$p < 0.01$; †$p < 0.05$ (Wilcoxon matched pairs test).

ponwerke, Germany) 0.010–6.0 mg/kg, or gepirone (Bristol-Myers-Squibb, USA) 0.025–4.0 mg/kg in animals pretreated with ICV 5,7-DHT or vehicle.

In the second set of experiments 4.0 mg/kg buspirone, 6.0 mg/kg ipsapirone, or 4.0 mg/kg gepirone was injected into the vehicle-infused controls pretreated with (−)-pindolol (Sandoz, Switzerland) 2.0 mg/kg.

Subcutaneous injections were given in a final volume of 1.0 ml/kg. The drugs were given 15 min apart in the interaction experiments. The azapirones and (−)-pindolol were dissolved in a small volume of glacial acetic acid and were diluted with distilled water; the pH was adjusted to 6. Fifteen minutes after SC injection a 6-h sleep recording was started at approximately 0800 h. At least 4 days were allowed to elapse between experiments to avoid long-lasting and rebound effects on sleep.

Statistics

The Kruskal–Wallis ANOVA by ranks was used for statistical comparison of four or more samples, with post hoc comparisons performed by the Wilcoxon matched pairs test.

TABLE 2

EFFECTS OF IPSAPIRONE ON WAKEFULNESS, LIGHT SLEEP, SLOW-WAVE SLEEP, AND REM SLEEP IN VEHICLE-TREATED RATS DURING 6-h SESSIONS

	0–2 h	3–4 h	5–6 h
Wakefulness			
Control	32.8 ± 11.2	23.6 ± 2.4	25.2 ± 3.8
Ipsapirone (mg/kg)			
0.010	50.0 ± 9.0	22.4 ± 4.8	26.0 ± 5.2
0.025	47.6 ± 4.2*	22.4 ± 3.0	29.2 ± 3.6
2.0	58.0 ± 8.8†	20.8 ± 2.8	25.4 ± 3.2
6.0	62.8 ± 3.6†	34.6 ± 9.2	24.0 ± 4.2
Light sleep			
Control	20.6 ± 1.2	16.6 ± 1.2	17.0 ± 2.0
Ipsapirone (mg/kg)			
0.010	14.4 ± 1.4†	12.6 ± 1.2	17.2 ± 2.2
0.025	15.0 ± 1.8†	15.8 ± 1.4	17.6 ± 1.4
2.0	11.2 ± 1.2†	11.0 ± 1.6	19.4 ± 1.2
6.0	15.0 ± 2.0*	11.8 ± 3.2	14.8 ± 1.0
Slow-wave sleep			
Control	61.4 ± 3.2	67.2 ± 3.2	66.4 ± 4.2
Ipsapirone (mg/kg)			
0.010	50.6 ± 7.2	74.0 ± 2.6	65.6 ± 4.2
0.025	54.2 ± 3.4	70.4 ± 2.6	63.8 ± 4.6
2.0	49.8 ± 8.1	82.2 ± 2.8	63.8 ± 4.0
6.0	42.2 ± 5.0*	77.4 ± 5.6	73.4 ± 3.0
REM sleep			
Control	5.2 ± 1.0	12.6 ± 2.0	11.4 ± 1.8
Ipsapirone (mg/kg)			
0.010	5.0 ± 2.0	11.0 ± 1.2	11.2 ± 0.6
0.025	3.2 ± 0.8	11.4 ± 1.0	9.4 ± 1.0
2.0	1.0 ± 1.0*	6.0 ± 1.6*	11.4 ± 1.4
6.0	0*	1.4 ± 1.0†	7.8 ± 3.0

All values are mean ± SEM in minutes. Seven animals were in each experimental group. *†Compared with control values: $*p < 0.05$; $†p < 0.01$ (Wilcoxon matched pairs test).

TABLE 3

EFFECTS OF GEPIRONE ON WAKEFULNESS, LIGHT SLEEP, SLOW-WAVE SLEEP, AND REM SLEEP IN VEHICLE-TREATED RATS DURING 6-h SESSIONS

	0–2 h	3–4 h	5–6 h
Wakefulness			
Control	33.0 ± 3.6	25.5 ± 2.2	27.4 ± 3.2
Gepirone			
0.025	46.0 ± 5.2*	27.4 ± 3.0	23.6 ± 4.4
0.100	48.0 ± 4.8†	23.6 ± 3.4	29.6 ± 5.4
2.0	66.8 ± 6.2†	24.0 ± 3.8	26.4 ± 3.2
4.0	75.0 ± 3.6†	23.2 ± 3.0	30.8 ± 4.6
Light sleep			
Control	20.4 ± 1.6	16.6 ± 1.2	18.0 ± 2.0
Gepirone (mg/kg)			
0.025	13.4 ± 1.8†	14.2 ± 1.4	13.8 ± 1.6
0.100	13.4 ± 2.6†	14.2 ± 2.4	14.8 ± 1.6
2.0	11.8 ± 1.4†	16.2 ± 1.4	18.0 ± 1.8
4.0	10.4 ± 1.4†	10.0 ± 1.4	15.8 ± 1.4
Slow-wave sleep			
Control	61.6 ± 2.6	67.0 ± 2.6	63.4 ± 3.8
Gepirone (mg/kg)			
0.025	56.4 ± 5.0	69.2 ± 2.4	67.2 ± 3.2
0.100	52.4 ± 4.0	68.2 ± 3.8	61.8 ± 5.8
2.0	41.4 ± 5.4†	71.6 ± 3.2	62.8 ± 2.2
4.0	34.6 ± 3.6†	82.0 ± 4.8	64.4 ± 4.2
REM sleep			
Control	5.0 ± 0.8	10.9 ± 1.8	11.2 ± 1.6
Gepirone (mg/kg)			
0.025	4.2 ± 0.8	9.2 ± 1.0	15.4 ± 2.0
0.100	6.2 ± 0.8	14.0 ± 1.4	13.8 ± 2.0
2.0	0†	8.2 ± 0.8	12.8 ± 1.6
4.0	0†	4.8 ± 1.2†	9.0 ± 1.0

All values are mean ± SEM in minutes. Eight animals were in each experimental group. *†Compared with control values: $*p < 0.05$; $†p < 0.01$ (Wilcoxon matched pairs test).

Biochemical Assays

Immediately after completion of experiments animals were killed by decapitation and each brain removed. Serotonin, 5-hydroxyindolacetic acid (5-HIAA), and norepinephrine levels were assayed in the raphe regions (pons and upper brain stem), hippocampus, cerebral cortex, and striatum using reverse-phase high pressure liquid chromatography with electrochemical detection (11).

RESULTS

Effects of Buspirone, Ipsapirone, or Gepirone in Vehicle-Infused Animals

Following the administration of 2.0–4.0 mg/kg buspirone, 0.025–6.0 mg/kg ipsapirone, or 0.025–4.0 mg/kg gepirone by SC route to the vehicle-infused controls, W was increased whereas LS, SWS, and REMS were significantly reduced (Tables 1, 2, and 3). Smaller doses of the azapirones were not effective in this respect. Effects on W, LS, and SWS after buspirone, ipsapirone, or gepirone were evident only during the first two recording hours. On the other hand, REMS re-

TABLE 4

EFFECTS OF 5,7-DIHYDROXYTRYPTAMINE ON MONOAMINES CONTENT IN BRAIN

	Serotonin (pg/mg)		5-HIAA (pg/mg)		Norepinephrine (pg/mg)	
Tissue	Control	Treated	Control	Treated	Control	Treated
Raphe	660.5 ± 81.8	39.0 ± 14.1*	591.2 ± 103.3	46.4 ± 17.7*	255.1 ± 52.3	217.6 ± 21.9
Cortex	678.4 ± 35.1	90.5 ± 19.2*	386.9 ± 12.1	62.4 ± 11.9*	303.5 ± 32.8	321.2 ± 15.8
Hippocampus	329.1 ± 9.0	7.8 ± 3.8*	309.4 ± 10.4	17.8 ± 4.8*	457.0 ± 27.3	458.4 ± 31.1
Striatum	438.8 ± 17.2	21.3 ± 2.3*	494.3 ± 21.3	85.8 ± 31.1*	109.2 ± 6.2	120.3 ± 10.9

Rats were sacrificed 40–45 days after 5,7-DHT or vehicle administration. All values are mean ± SEM. Six animals were in each experimental group.

*$p < 0.001$ (two tailed t-test).

mained significantly reduced after the highest dose(s) of either compound during the second 2-h period after treatment.

Effects of Buspirone, Ipsapirone, or Gepirone in 5,7-DHT-Infused Animals

5,7-DHT-treated animals showed a marked and significant serotonin and 5-HIAA depletion in the raphe regions of the pons and upper brain stem, cerebral cortex, hippocampus, and striatum. In contrast, norepinephrine levels remained almost unchanged (Table 4).

Values corresponding to sleep-wake states after 5,7-DHT did not differ significantly compared to the vehicle-treated group. Subcutaneous administration of buspirone, ipsapirone, or gepirone to the 5,7-DHT-treated animals induced changes similar to these described in the vehicle-treated group. Accordingly, W was increased whereas LS, SWS and REMS were reduced (Tables 5, 6, and 7). However, compared to the vehicle-infused controls, values corresponding to W after the 4.0-mg/kg dose of buspirone were significantly smaller in the 5,7-DHT-lesioned animals.

Comparison between the lesioned and the control groups revealed no temporal differences in the respective buspirone- and gepirone-induced changes of non-REMS (LS + SWS) and W.

Effects of Buspirone, Ipsapirone, or Gepirone in (−)-Pindolol-Pretreated Animals

As can be seen from Figs. 1 and 3, (−)-pindolol (2.0 mg/kg) effectively antagonized the increase of W and decrease of non-REM sleep induced by buspirone (4.0 mg/kg) and gepirone (4.0 mg/kg). Nevertheless, buspirone- or gepirone-related suppression of REMS was not prevented by pretreatment with (−)-pindolol. As regards ipsapirone, (−)-pindolol failed to reverse its effects on sleep and W (Fig. 2).

DISCUSSION

Acute injection of relatively large amounts of the azapirones buspirone, ipsapirone, or gepirone in the vehicle-infused controls significantly increased W and reduced non-REM sleep and REMS. No decrease of W and/or increase of SWS was observed when drugs were given in relatively low dosages. In this respect, they contrast with 8-OH-DPAT, which induces biphasic effects such that low doses decrease W and increase SWS whereas higher doses induce opposite effects (23). Decreased W and increased SWS after low doses of 8-OH-DPAT have been related to selective activation of the somatodendritic autoreceptor, whereas opposite effects have been ascribed to stimulation of postsynaptic receptors (24). Differ-

TABLE 5

EFFECTS OF BUSPIRONE ON WAKEFULNESS, LIGHT SLEEP, SLOW-WAVE SLEEP, AND REM SLEEP IN 5,7-DIHYDROXYTRYPTAMINE-TREATED RATS DURING 6-h SESSIONS

	0–2 h	3–4 h	5–6 h
Wakefulness			
Control	45.0 ± 8.0	27.0 ± 4.2	25.4 ± 6.4
Buspirone (mg/kg)			
0.010	49.0 ± 4.4	30.6 ± 2.4	28.8 ± 6.8
0.025	45.6 ± 5.4	21.0 ± 2.4	39.8 ± 6.0
2.0	79.6 ± 3.2*	27.8 ± 3.6	19.8 ± 3.8
4.0	80.8 ± 5.0*	29.8 ± 6.4	24.0 ± 6.2
Light sleep			
Control	20.0 ± 2.4	18.0 ± 3.6	20.0 ± 2.6
Buspirone (mg/kg)			
0.010	15.0 ± 1.4	15.0 ± 3.0	13.2 ± 0.8
0.025	16.2 ± 1.7	17.6 ± 2.2	14.6 ± 1.0
2.0	7.8 ± 1.4*	12.4 ± 1.4	13.8 ± 2.0
4.0	11.4 ± 1.9	16.4 ± 1.4	19.8 ± 2.2
Slow-wave sleep			
Control	50.0 ± 8.8	64.2 ± 2.6	62.8 ± 5.8
Buspirone (mg/kg)			
0.010	50.6 ± 3.8	64.2 ± 4.2	66.6 ± 6.0
0.025	52.0 ± 2.8	70.4 ± 3.6	57.0 ± 4.8
2.0	32.3 ± 3.2	73.8 ± 3.4	69.2 ± 3.0
4.0	27.8 ± 5.4*	67.6 ± 5.8	65.8 ± 6.4
REM sleep			
Control	5.0 ± 0.8	10.8 ± 1.8	11.8 ± 1.6
Buspirone (mg/kg)			
0.010	5.4 ± 0.8	10.2 ± 1.4	11.4 ± 1.6
0.025	6.2 ± 1.8	11.0 ± 2.4	8.6 ± 1.4
2.0	0.3 ± 0.3*	6.0 ± 3.0	17.2 ± 3.0
4.0	0*	6.0 ± 6.0	10.4 ± 2.0

All values are mean ± SEM in minutes. Six animals were in each experimental group. *Compared with control values: $p < 0.05$ (Wilcoxon matched pairs test).

TABLE 6

EFFECTS OF IPSAPIRONE ON WAKEFULNESS, LIGHT SLEEP, SLOW-WAVE SLEEP, AND REM SLEEP IN 5,7-DIHYDROXYTRYPTAMINE-TREATED RATS DURING 6-h SESSIONS

	0–2 h	3–4 h	5–6 h
Wakefulness			
Control	38.4 ± 5.2	27.0 ± 4.2	25.0 ± 6.4
Ipsapirone (mg/kg)			
0.010	51.2 ± 4.8	31.6 ± 3.4	27.8 ± 4.4
0.025	45.6 ± 4.8	32.4 ± 4.8	26.0 ± 3.8
2.0	58.6 ± 3.4*	28.0 ± 5.0	36.0 ± 3.4
6.0	69.4 ± 4.4*	21.4 ± 6.8	32.6 ± 5.4
Light sleep			
Control	20.0 ± 2.4	18.0 ± 3.6	20.4 ± 2.8
Ipsapirone (mg/kg)			
0.010	11.8 ± 2.0	15.8 ± 1.2	16.2 ± 1.8
0.025	13.2 ± 2.2	10.4 ± 1.8	17.4 ± 1.0
2.0	10.0 ± 2.0	10.8 ± 1.0	18.0 ± 2.4
6.0	14.2 ± 2.0	14.2 ± 0.4	19.6 ± 1.6
Slow-wave sleep			
Control	56.8 ± 6.4	64.6 ± 2.6	62.8 ± 5.8
Ipsapirone (mg/kg)			
0.010	53.2 ± 5.0	63.6 ± 2.4	65.2 ± 4.4
0.025	57.6 ± 5.0	67.8 ± 3.6	66.2 ± 3.4
2.0	50.6 ± 6.0	76.2 ± 5.0	54.8 ± 6.0
6.0	36.0 ± 4.2*	81.4 ± 6.4*	59.0 ± 3.4
REM sleep			
Control	4.8 ± 0.8	10.4 ± 2.0	11.8 ± 1.6
Ipsapirone (mg/kg)			
0.010	3.8 ± 1.2	9.0 ± 2.6	10.8 ± 1.2
0.025	3.6 ± 1.6	9.4 ± 1.6	10.4 ± 2.4
2.0	0.8 ± 0.4*	5.0 ± 1.8*	11.2 ± 1.6
6.0	0.4 ± 0.4*	3.0 ± 1.0*	8.8 ± 1.6

All values are mean ± SEM in minutes. Six animals were in each experimental group. *Compared with control values: $p < 0.05$ (Wilcoxon matched pairs test).

ences in the effect of partial agonists and 8-OH-DPAT on sleep variables could depend on the former acting as full agonists at presynaptic sites and as antagonists in some serotonin projecting areas, such as the cortex (6). Thus, decreased firing rate of 5-HT neurons and the resultant reduction of W and/or increase of SWS after small doses of the azapirones would be tentatively prevented by the release of a facilitatory feedback mechanism.

Subcutaneous administration of the azapirones to the 5,7-DHT-treated group led to an increase of W and a reduction of sleep. However, compared to the vehicle-infused animals, values corresponding to W after the 4.0-mg/kg dose of buspirone were significantly smaller in the 5,7-DHT-lesioned animals. The discrepancy could be related to the moderate difference between the control means (vehicle-infused = 37.6 ± 5.6 min; 5,7-DHT-treated = 45.0 ± 8.0 min). Although these results are not significantly different, they could cause a bias in favor of the vehicle-treated group. In general, the increment in W time induced by CNS stimulants is related inversely to the control values.

In addition, it should be considered that buspirone shows an antagonist action on presynaptic dopaminergic neurotransmission (33). Moreover, as shown recently by Callaway et al. (2) and Schmidt et al. (29), 5-HT_1 and 5-HT_2 receptors exert a facilitatory role on dopaminergic function, and their blockade attenuates the presynaptic D_2 receptor-mediated increase in dopamine synthesis and release. Because a good deal of evidence favors a facilitatory role of dopamine antagonists on W through a blockade of inhibitory presynaptic D_2 receptors (21), it could be tentatively proposed that administration of 5,7-DHT would decrease the dopamine-dependent increase of W after buspirone administration, in a way similar to the pharmacological blockade of 5-HT receptors.

The finding that the azapirones-induced increase of W and decrease of sleep were still present in the 5,7-DHT-lesioned animals further supports their dependence on postsynaptic 5-HT_{1A} receptors. The latter could be tentatively located in the hippocampus where azapirones act as agonists (6). In other words, differences in the effect of full agonists (8-OH-DPAT) and partial agonists (azapirones) on sleep–wake states could be related to variations in the functional response of different postsynaptic 5-HT_{1A} receptor populations to the latter.

The β-adrenoceptor antagonist (−)-pindolol, which blocks

TABLE 7

EFFECTS OF GEPIRONE ON WAKEFULNESS, LIGHT SLEEP, SLOW-WAVE SLEEP, AND REM SLEEP IN 5,7-DIHYDROXYTRYPTAMINE-TREATED RATS DURING 6-h SESSIONS

	0–2 h	3–4 h	5–6 h
Wakefulness			
Control	38.4 ± 5.2	27.0 ± 4.4	25.0 ± 6.4
Gepirone (mg/kg)			
0.025	50.8 ± 5.6	24.0 ± 3.6	33.2 ± 7.4
0.100	47.0 ± 4.4	26.0 ± 5.8	34.6 ± 4.0
2.0	62.0 ± 6.2	21.0 ± 6.0	18.0 ± 2.6
4.0	74.8 ± 8.5*	24.2 ± 2.8	34.8 ± 7.0
Light sleep			
Control	20.0 ± 2.4	18.0 ± 3.8	20.4 ± 2.8
Gepirone (mg/kg)			
0.025	17.6 ± 1.0	13.8 ± 0.8	19.2 ± 2.2
0.100	15.2 ± 1.8*	15.2 ± 0.8	16.2 ± 1.4
2.0	12.6 ± 1.8*	15.6 ± 2.0	17.4 ± 1.4
4.0	9.8 ± 1.2*	14.6 ± 0.8	18.2 ± 2.4
Slow-wave sleep			
Control	56.8 ± 6.4	64.6 ± 2.6	62.8 ± 5.8
Gepirone (mg/kg)			
0.025	48.2 ± 4.8	70.2 ± 3.8	55.4 ± 8.4
0.100	54.6 ± 4.8	68.4 ± 5.0	58.0 ± 3.6
2.0	44.8 ± 5.8	73.6 ± 6.6	71.4 ± 1.8
4.0	34.2 ± 5.6*	72.0 ± 3.2	54.6 ± 5.6
REM sleep			
Control	4.8 ± 0.8	10.4 ± 2.0	11.8 ± 1.6
Gepirone (mg/kg)			
0.025	3.4 ± 1.0	12.0 ± 1.6	12.2 ± 2.0
0.100	3.4 ± 1.2	10.4 ± 2.8	11.2 ± 1.6
2.0	0.6 ± 0.6*	9.8 ± 1.6	13.2 ± 1.8
4.0	1.2 ± 1.2*	9.2 ± 1.4	12.4 ± 2.6

All values are mean ± SEM in minutes. Six animals were in each experimental group. *Compared with control values: $p < 0.05$ (Wilcoxon matched pairs test).

pre- and postsynaptic 5-HT_{1A} receptors, antagonized the actions of buspirone and gepirone on W and non-REM sleep, which also adds to the involvement of 5-HT_{1A} receptors in their disruptive effect on sleep and W. As regards ipsapirone, (−)-pindolol was ineffective in this respect. One possibility is that higher amounts of (−)-pindolol could be required to prevent the effects of ipsapirone. Another possibility is that mechanisms not related to the 5-HT_{1A} receptor are partly responsible for the increase of W (10,12).

REM sleep was suppressed after administration of the azapirones in both the vehicle-infused and the 5,7-DHT-treated animals, and this effect was not prevented by (−)-pindolol. REM sleep is a very "fragile" entity in the rat and can be disrupted by pharmacological agents with quite different mechanisms of action. Also, changes in body temperature induced by CNS-acting drugs can interfere with REMS occurrence. In this respect, ipsapirone given in doses of 2.5–10.0 mg/kg IP did not significantly modify body temperature in the rat (19). Thus, a strict relationship between administration of the azapirones, REMS suppression, and changes in body temperature cannot be easily acknowledged.

It could be argued that W increase after azapirones administration is related to the 5-HT behavioral syndrome, which is also resistant to treatment with the neurotoxin 5,7-DHT (31). Although buspirone (50.0–200.0 mg/kg, IP) and ipsapirone (10.0–80.0 mg/kg, IP) failed to induce the serotonin syndrome in nonlesioned rats, gepirone (10.0–50.0 mg/kg, IP) was active in this test (5,7). However, doses of gepirone given in our study (0.025–4.0 mg/kg, SC) were far below the ones inducing the 5-HT behavioral syndrome, which tends to discard its participation in their disruption of sleep and W.

Similar sleep-disrupting effects for buspirone have been described in insomniac patients (20). Thus, on the first night of buspirone (10 mg) administration W after sleep onset was significantly increased, and for the first 3 drug nights, this value and total wake time were increased moderately.

Onset of the anxiolytic action of buspirone and gepirone typically occurs after 2–3 weeks of administration (3,27). In spite of that, there is no evidence of tolerance to the stimulant action of an acute systemic challenge dose of buspirone or ipsapirone after repeated treatment with high doses. The postsynaptic 5-HT_{1A} receptor also appears to be unaffected upon chronic agonist treatment (1,32). However, in spite of buspirone, ipsapirone and gepirone having a relatively low affinity for other 5-HT receptor sites, following their chronic use, a decrease in the number of 5-HT_2 and 5-HT_3 receptors has been reported in the rat brain (1,5,28,32). Thus, available evidence tends to indicate that different receptor mechanisms could be involved in the stimulant and anxiolytic effects of the azapirones.

In conclusion, the present results show that acute administration of the 5-HT_{1A} receptor partial agonists buspirone, ipsapirone, and gepirone increase W and decrease sleep. The sleep disruption induced by the azapirones was still present in animals lesioned with the serotonin neurotoxin 5,7-DHT. Moreover, (−)-pindolol prevented the effects of buspirone and gepirone on W and SWS.

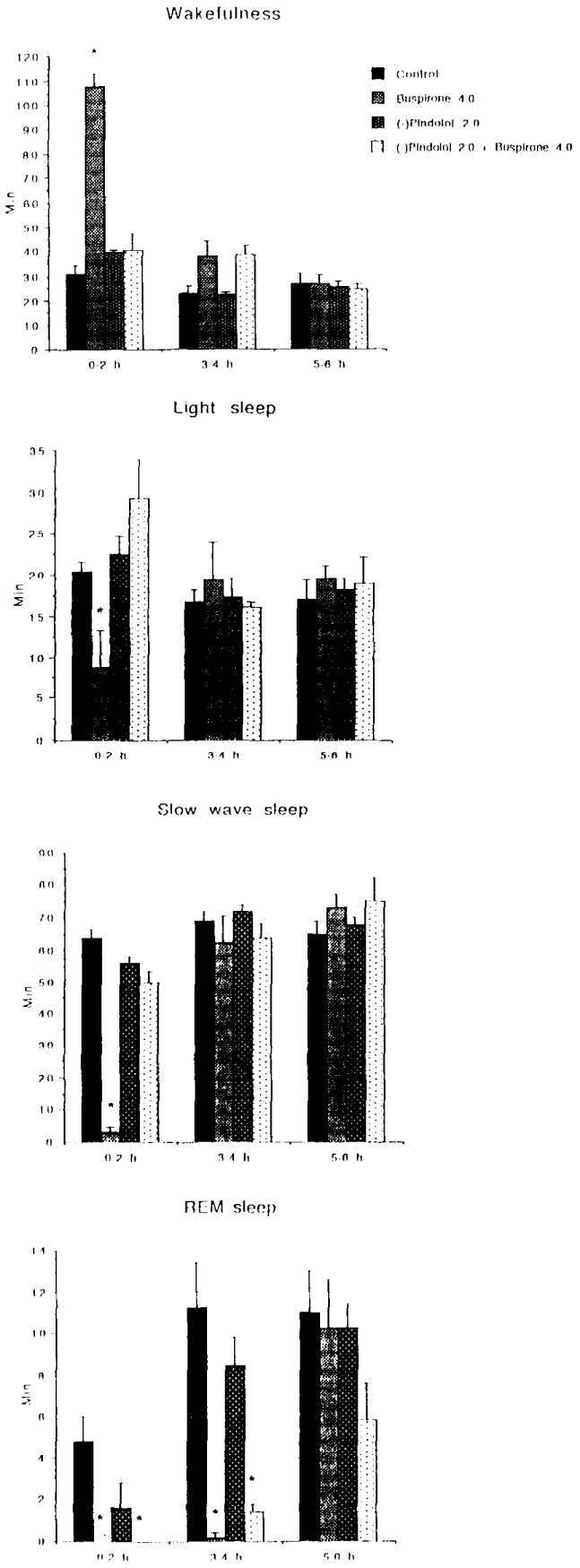

FIG. 1. The effect of (−)-pindolol pretreatment on the buspirone-induced increase in wakefulness and decrease in light sleep, slow-wave sleep, and REM sleep during 6-h sessions. All values are the means ± SEM (min). Six animals were in each experimental group. The doses are in mg/kg. Compared with control values: *$p < 0.05$ (Wilcoxon matched pairs test).

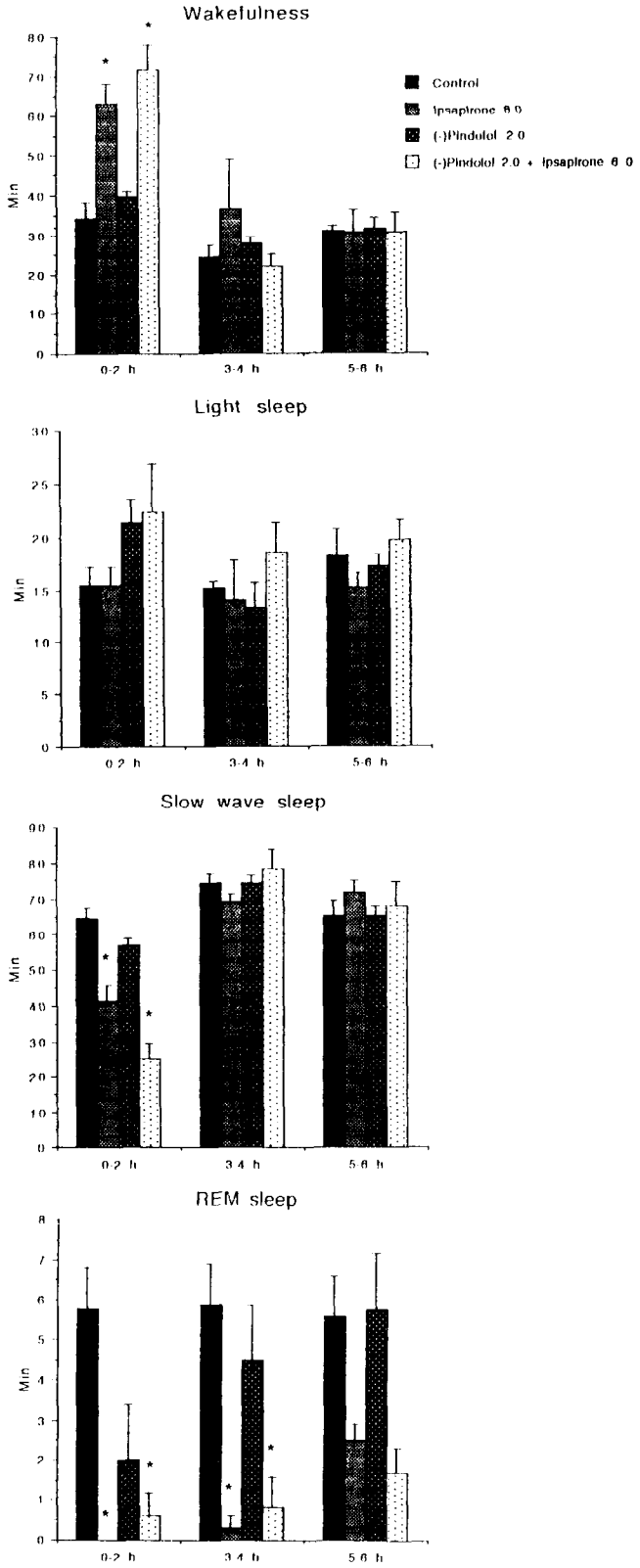

FIG. 2. The effect of (−)-pindolol pretreatment on the ipsapirone-induced increase in wakefulness and decrease in slow-wave sleep and REM sleep during 6-h sessions. Each experimental group contained six animals. Compared with control values: $*p < 0.05$ (Wilcoxon signed rank test).

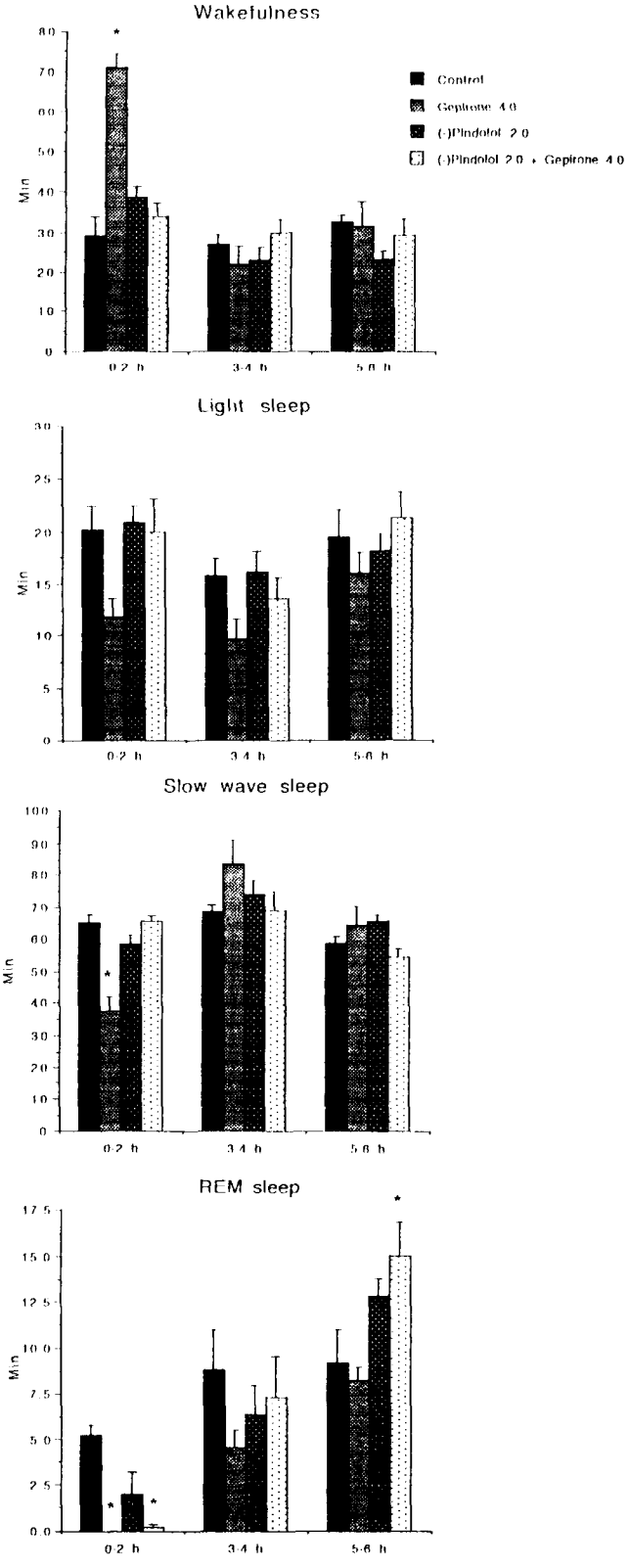

FIG. 3. The effect of (−)-pindolol pretreatment on the gepirone-induced increase in wakefulness and decrease in slow-wave sleep and REM sleep. Six animals were in each experimental group. Compared with control values: $*p < 0.05$ (Wilcoxon signed rank test).

REFERENCES

1. Barrett, J. E.; Vanover, K. E. 5-HT receptors as targets for the development of novel anxiolytic drugs:models, mechanisms and future directions. Psychopharmacology (Berlin) 112:1-12; 1993.
2. Callaway, C. W.; Rempel, N.; Peng, R. Y.; Geyer, M. A. Serotonin 5-HT-like receptors mediate hyperactivity in rats induced by 3,4-methylenedioxy-methamphetamine. Neuropsychopharmacology 7:113-127; 1992.
3. Csanalosi, I.; Schweizer, E.; Case, W. G.; Rickels, K. Gepirone in anxiety: A pilot study. J. Clin. Psychopharmacol. 7:31-33; 1987.
4. Eison, A. S.; Eison, M. S.; Stanley, M.; Riblet, L. A. Serotonergic mechanisms in the behavioral effects of buspirone and gepirone. Pharmacol. Biochem. Behav. 24:701-707; 1986.
5. Eison, A. S.; Yocca, F. D. Reduction in cortical 5-HT_2 receptor sensitivity after continuous gepirone treatment. Eur. J. Pharmacol. 111:389-392; 1985.
6. Fletcher, A.; Cliffe, I. A.; Dourish, C. T. Silent 5-HT_{1A} receptor antagonist: Utility as research tools and therapeutic agents. Trends Pharmacol. Sci. 14:441-448; 1993.
7. Glaser, T.; Dompert, W. U.; Schuurman, T.; Spencer, D. G.; Traber, J. Differential pharmacology of the novel 5-HT_{1A} receptor ligands 8-OH-DPAT, BAY R 1531 and ipsapirone. In: Dourish, C. T.; Alhenius, S.; Hutson, P. H., eds. Brain 5-HT_{1A} receptors. Chichester: Ellis Horwood; 1987:106-119.
8. Glaser, T.; De Vry, J. Neurobiology of 5-HT_{1A} receptors. In: Stahl, M.; Gastpar, J. M.; Keppel Hesselink, J. M.; Traber, J., eds. Serotonin$_{1A}$ receptors in depression and anxiety. New York: Raven Press; 1992:35-53.
9. Glaser, T.; Greuel, J. M.; Horvath, E.; Schreiber, R.; De Vry, D. Differentiation of 8-OH-DPAT and ipsapirone in rat models of 5-HT_{1A} receptor function. In: Fozard, J. R.; Saxena, P. R., eds. Serotonin: Molecular biology, receptors and functional effects. Basel: Birkhauser Verlag; 1991:460-472.
10. Gower, A. J.; Tricklebank, M. D. α_2-adrenoceptor antagonist activity may account for the effects of buspirone in an anticonflict test in the rat. Eur. J. Pharmacol. 155:129-137; 1988.
11. Hallman, H.; Jonsson, G. Neurochemical studies on central dopamine neurons. Regional characterization of dopamine turnover. Med. Biol. 62:198-202; 1984.
12. Hamon, M.; Fattaccini, C. M.; Adrien, J.; Gallissont, M. C.; Martin, P.; Gozlan, H. Alteration of central serotonin and dopamine turnover in rats treated with ipsapirone and other 5-hydroxytryptamine$_{1A}$ agonists with potential anxiolytic properties. J. Pharmacol. Exp. Ther. 246:745-752; 1988.
13. Hoyer, D. Functional correlates of serotonin 5-HT_1 recognition sites. J. Recept. Res. 8:59-81; 1988.
14. Hoyer, D. 5-hydroxytryptamine receptors and effector coupling mechanisms in peripheral tissues. In: Fozard, V., ed. The peripheral actions of 5-hydroxytryptamine. Oxford: Oxford University Press; 1989:72-99.
15. Hoyer, D.; Engel, G.; Kalkman, H. O. Molecular pharmacology of 5-HT_1 and 5-HT_2 recognition sites in rat and pig brain membranes: Radioligand binding studies with (^{3}H)-5HT, (^{3}H)-8-OH-DPAT, (−)[^{125}]-iodo-cyanopindolol, (^{3}H)-mesulergine and (^{3}H)-ketanserin. Eur. J. Pharmacol. 118:13-23; 1985.
16. Humphrey, P. P. A.; Hartig, P.; Hoyer, D. A proposed new nomenclature for 5-HT receptors. Trends Pharmacol. Sci. 14: 233-236; 1993.
17. Kalkman, H. O.; Soar, J. Determination of the 5-HT receptor subtype involved in 8-OH-DPAT-induced hyperlocomotion: Potential difficulties arising from inadequate pharmacological tools. Eur. J. Pharmacol. 191:383-390; 1990.
18. Lerman, J. A.; Kaitin, K. I.; Dement, W. C.; Peroutka, S. J. The effects of buspirone on sleep in the rat. Neurosci. Lett. 72:64-68; 1986.
19. Maj, J.; Chojnacka-Wójcik, E.; Tatarczynska, E.; Klodzinska, A. Central action of ipsapirone, a new anxiolytic drug, on serotoninergic, noradrenergic and dopaminergic functions. J. Neural Transm. 70:1-17; 1987.
20. Manfredi, R. L.; Kales, A.; Vgontzas, A. N.; Bixler, E. O.; Isaac, M. A.; Falcone, C. M. Buspirone: Sedative or stimulant effect? Am. J. Psychiatry 148:1213-1217; 1991.
21. Monti, J. M.; Hawkins, M.; Jantos, H.; Labraga, P.; Fernández, J. Biphasic effects of dopamine D_2 receptor agonists on sleep and wakefulness in the rat. Psychopharmacology (Berlin) 95:395-400; 1988.
22. Monti, J. M.; Orellana, C.; Boussard, M.; Jantos, H.; Labraga, P.; Olivera, S.; Alvariño, F. 5-HT receptor agonists 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and 8-OH-DPAT increase wakefulness in the rat. Biogenic Amines 7:145-151; 1990.
23. Monti, J. M.; Jantos, H. Dose-dependent effects of the 5-HT_{1A} receptor agonist 8-OH-DPAT on sleep and wakefulness in the rat. J. Sleep Res. 1:169-175; 1992.
24. Monti, J. M. Jantos, H.; Silveira, R.; Reyes-Parada, M.; Scorza, C.; Prunell, G. Depletion of brain serotonin by 5,7-DHT: Effects on the 8-OH-DPAT-induced changes of sleep and waking in the rat. Psychopharmacology (Berlin) 115:273-277; 1994.
25. Olivier, B.; Mos, J.; Tulp, M. Th. M., van der Poel, A. M. Animal models of anxiety and aggression in the study of serotonergic agents. In: Langer, S. Z.; Brunello, N.; Racagni, G.; Mendlewicz, J., eds. Serotonin receptor subtypes: Pharmacological significance and clinical implications. Int. Acad. Biomed. Drug Res. 1:67-79; 1992.
26. Paxinos, G.; Watson, C. The rat brain, 2nd ed. Sidney: Academic Press; 1986.
27. Rickels, K.; Weisman, K.; Norstad, N.; Singer, M.; Stoltz, P.; Brown, A.; Danton, J. Buspirone and diazepam in anxiety: A controlled study. J. Clin. Psychiatry 43:81-86; 1982.
28. Schechter, L. E.; Bolanos, F. J.; Gozlan, H.; Lanfumey, L.; Haj-Dahmane, S.; Laporte, A. -M.; Fattaccini; C.-M.; Hamon, M. Alterations of central serotoninergic and dopaminergic neurotransmission in rats chronically treated with ipsapirone: Biochemical and electrophysiological studies. J. Pharmacol. Exp. Ther. 255:1335-1347; 1990.
29. Schmidt, C. J.; Fadayel, G. M.; Sullivan, C. K.; Taylor, V. L. 5-HT_2 receptors exert a state-dependent regulation of dopaminergic function: Studies with MDL 100,907 and the amphetamine analogue, 3,4-methylenedioxy-methanphetamine. Eur. J. Pharmacol. 223:65-71; 1992.
30. Sharp, T.; Backus, L. I.; Hjorth, S.; Bramwell, S. R.; Grahame-Smith, D. G. Further investigations of the in vivo pharmacological properties of the putative 5-HT_{1A} antagonist BMY 7378. Eur. J. Pharmacol. 176:331-340; 1990.
31. Smith, L. M.; Peroutka, S. J. Differential effects of 5-hydroxytryptamine$_{1A}$ selective drugs on the 5-HT behavioral syndrome. Pharmacol. Biochem. Behav. 24:1513-1519; 1986.
32. Söderpalm, B.; Lundin, B.; Hjorth, S. Sustained 5-hydroxytryptamine release-inhibitory and anxiolytic action of the partial 5-HT_{1A} receptor agonist buspirone, after prolonged chronic administration. Eur. J. Pharmacol. 239:69-73; 1993.
33. Taylor, D. P. Buspirone, a new approach to the treatment of anxiety. FASEB J. 2:2445-2452; 1988.
34. Taylor, D. P.; Hyslop, D. K. Chronic administration of buspirone downregulates 5-HT_2 receptor binding sites. Drug Dev. Res. 24:93-105; 1991.
35. Tissier, M. -H.; Lainey, E.; Fattaccini, C. -M.; Hamon, M.; Adrien, J. Effects of ipsapirone, a 5-HT_{1A} agonist, on sleep/wakefulness cycles: Probable postsynaptic action. J. Sleep Res. 2: 103-109; 1993.
36. Vergé;, D.; Daval. G.; Marcinkiewicz, M.; Patey, A.; El Mestikawy, S.; Gozlan, H.; Hamon, M. Quantitative autoradiography of multiple 5-HT_1 receptor subtypes in the brain of control or 5,7-dihydroxytryptamine-treated rats. J. Neurosci. 6:3474-3482; 1986.
37. Zifa, E.; Fillion, G. 5-Hydroxytryptamine receptors. Pharmacol. Rev. 44:401-458; 1992.