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Sleep and Waking during Acute Histamine H_3 Agonist BP 2.94 or H_3 Antagonist Carboperamide (MR 16155) Administration in Rats

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The present study evaluated the effects of histamine H_3 receptor agonist BP 2.94 or H_3 receptor antagonist carboperamide (MR 16155) given by oral route on sleep and waking in rats surgically prepared for long-term recordings.

BP 2.94 produced a significant increase of slow-wave sleep (SWS) that was related to slight decreases of waking, light sleep, and REM sleep. Carboperamide significantly increased waking and decreased SWS and REM sleep. Pretreatment with carboperamide prevented the effect of BP 2.94 on SWS.

It is suggested that the effects of BP 2.94 or carboperamide on sleep and waking could depend on changes in the availability of histamine at the postsynaptic H_1 receptor. Alternatively, activation or blockade of the H_3 heteroreceptors found in the central catecholamine, indolamine, and acetylcholine nerve endings could inhibit or increase the release of noradrenaline, serotonin, dopamine, and acetylcholine. This would secondarily result in changes of sleep variables. ***[Neuropsychopharmacology 15:31–35, 1996]***

KEY WORDS: *Histamine H_3 receptor; BP 2.94; Carboperamide; Sleep waking; REM sleep*

Histamine-related functions in the central nervous system (CNS) are regulated at postsynaptic sites by the H_1 and H_2 receptors. Neuroanatomical, neurochemical, and neuropharmacological evidence presently indicates a role for histamine in the control of the waking state (see Monti 1993 for review). In this respect the H_1 receptor plays a predominant role. Thus, activation of the H_1 receptor by the highly selective agonist 2-(3-trifluoromethylphenyl)histamine increases waking, whereas the antagonists pyrilamine, diphenhydramine, and chlorpheniramine produce opposite effects (Lin et al. 1988; Monti et al. 1994).

The histamine H_3 receptor shows the features of a presynaptic autoreceptor, mediating the synthesis and release of histamine. The distribution of the H_3 receptor in the rat brain is highly heterogeneous and not exactly the same as that of histaminergic terminals, which is in accordance with its presence in nonhistaminergic nerve endings (Schlicker et al. 1989, 1993). In rodents the H_3 receptor is found in the cerebral cortex, structures corresponding to the limbic system (hippocampus, amygdala), the striatum and nucleus accumbens, the thalamus, the hypothalamus, the mesencephalon, and the lower brain stem (Schwartz et al. 1991; Arrang et al. 1992). In the human brain H_3 receptors predominate in the basal ganglia, mainly the globus pallidus (Martinez-Mir et al. 1990)

Studies aimed at determining the role of the H_3- receptor on sleep and waking have made use of the selective agonist (R)-α-methylhistamine and of the the antagonist thioperamide (Arrang et al. 1987; Lipp et al. 1992).

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(R)-α-methylhistamine injected bilaterally into the premammillary area of the rat, where histamine immunoreactive neurons are located, increased slow-wave sleep (SWS), whereas waking and REM sleep were reduced. No significant effects were observed when (R)-α-methylhistamine was administered intraperitoneally, which could be related to its poor penetration through the blood-brain barrier (Monti et al. 1991). Oral administration of the H_3 receptor agonist in a 10-mg/kg dose caused a significant increase of deep SWS in cats (Lin et al. 1990). On the other hand, thioperamide increased waking and decreased SWS and REM sleep in rats and cats. Pretreatment with thioperamide prevented the effect of (R)-α-methylhistamine on SWS and waking (Lin et al. 1990; Monti et al. 1991).

In order to increase (R)-α-methylhistamine penetration through the blood-brain barrier, Stark et al. (1992) designed a series of azomethine derivatives that are highly lipophilic and behave as prodrugs of the H_3 receptor. Using cortical [^{3}H]-histamine release and [^{3}H]-pyrilamine binding as tests for K_i determinations, it was observed that one of them, BP 2.94 [(R)-(−)-2-N-1-(1H-imidazol-4-yl)-2-propyl-iminophenylmethyl–phenol] shows affinity values (K_i in nM) for the H_1 and H_3 receptors of 1,000 and 3 [value of (R)-α-methylhistamine, the active constituent], respectively. Moreover, using cerebral t-methylhistamine level as test for ED_{50} determination, the value corresponding to BP 2.94 amounted to 4. The H_3 antagonist carboperamide [1-(octanoyl)-4-(1H-imidazolyl-4-piperidine) fumarate] became recently available (Ligneau et al. 1994; Schunack and Stark 1994). Compared to thioperamide, it crosses the blood-brain barrier to a similar extent and shows affinity values (K_i in nM) for the H_1 and H_3 receptors of 6,000 and 20, respectively. The ED_{50} (in mg/kg) shows a value of 7.

The present study was designed to quantify the effect of the H_3 receptor agonist BP 2.94 on sleep and waking in the rat. In addition, we examined the potential of carboperamide against BP 2.94-induced changes of sleep variables.

METHODS

Animals

Nine adult male rats of Wistar strain (range of body weight at start of experiment 320–350 g) were implanted under pentobarbital anesthesia (40 mg/kg, I.P.) with Nichrome(R) electrodes for chronic sleep recording from the frontal and occipital cortex and from the dorsal neck musculature. After surgery the rats were housed individually in a temperature-controlled room (20 ± 1°C), under a 12 hour light-dark cycle (lights on at 7:00 A.M.). Food and water were available ad libitum. At least 10 days were allowed for the rats to adapt to the implant after surgery. Rats were adapted to connection of the cable for at least four 10-hour sessions before drug administration began.

The scoring of the polygraph records was made in 20-second periods according to previously described criteria (Monti et al. 1988) for waking, light sleep, SWS, and REM sleep. Slow wave sleep latency (SWSL, from the beginning of the recording to the first 1-minute period of SWS) and REM sleep latency (from the first 1-minute period of SWS to REM sleep onset) were also determined.

Pharmacological Procedure

We studied the effects of BP 2.94 (Bioprojet, France), 3, 10, 20, and 30 mg/kg orally, in the first set of experiments. The doses are expressed in prodrug. However, if we consider that BP 2.94 and (R)-α-methylhistamine have molecular weights of 305.4 and 125.1, respectively (ratio between both molecular weights = 2.44), the actual doses of the active constituent amount to 1.22, 4.09, 8.18, and 12.2 mg/kg. In the second set of experiments carboperamide (Bioprojet, France), 3, 10, 20, and 30 mg/kg, was also given by oral route. In the third set of experiments BP 2.94, 30 mg/kg, was administered to animals pretreated with carboperamide, 30 mg/kg. The drugs were suspended in a 1% mixture of carboxymeth-

Table 1. Effects of BP 2.94 on Sleep and Waking

Treatment	W	LS	SWS	REMS	SWSL	REMSL
Control	90.2 ± 5.1	50.8 ± 7.3	178.4 ± 8.2	40.6 ± 3.0	23.1 ± 6.1	37.4 ± 6.6
BP 2.94						
3	94.0 ± 5.9	51.0 ± 6.6	174.0 ± 7.3	41.0 ± 5.0	18.4 ± 3.1	46.7 ± 9.1
10	90.2 ± 7.4	49.0 ± 4.6	185.4 ± 6.0	35.4 ± 4.8	10.9 ± 2.1	57.9 ± 12.4
20	82.3 ± 6.4	38.0 ± 5.0	201.6 ± 5.6**	38.1 ± 3.8	11.9 ± 2.9	37.2 ± 5.2
30	83.2 ± 7.0	37.8 ± 5.9	205.4 ± 6.9**	33.6 ± 2.8	16.9 ± 4.4	45.3 ± 7.7

W = waking; LS = light sleep; SWS = slow wave sleep; REMS = REM sleep; SWSL = slow-wave sleep latency; REMSL = REM sleep latency. All the values are the means ± SEM (minutes). Nine animals were in each experimental group. The doses are in mg/kg. Statistical comparison with control values:; ** $p < .01$ (Newman-Keuls test).

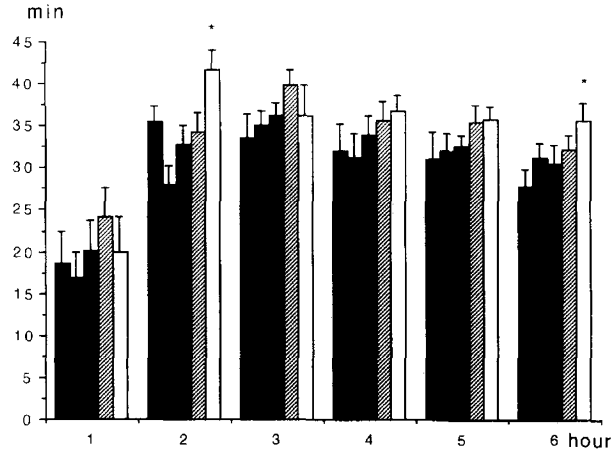

Figure 1. Hourly amounts of SWS during the first 6 hours following administration of BP 2.94 (3–30 mg/kg). Abscissae: Time in hours. Ordinate: Mean amount in minutes during SWS. All values are means ± SEM. Nine animals were in each experimental group. Slow-wave sleep was significantly increased during the second and sixth hours. *$p < .05$ (Newman-Keuls test). *Solid black bars,* controls; *dark cross-hatched bars,* BP3; *solid grey bars,* BP10; *light cross-hatched bars;* BP20; *open bars,* BP30.

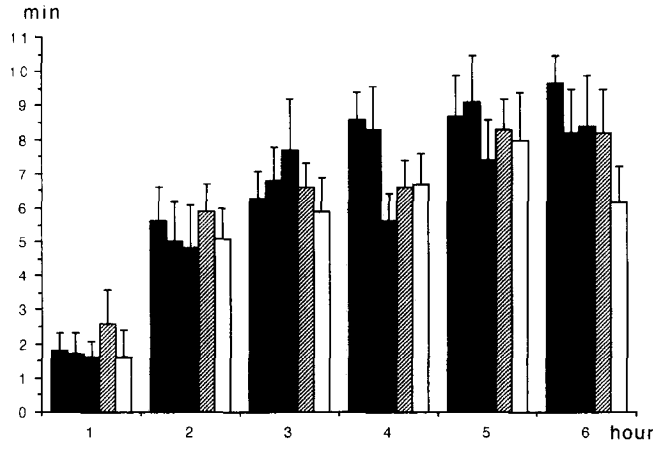

Figure 2. Hourly amounts of REM sleep during the first 6 hours following administration of BP 2.94 (3–30 mg/kg). Abscissae: Time in hours. Ordinate: Mean amount in minutes during REM sleep. REM sleep was not significantly modified during the first 6 recording hours. *Solid black bars,* controls; *dark cross-hatched bars,* BP 3; *solid grey bars,* BP 10; *light cross-hatched bars,* BP 20; *open bars,* BP 30.

ylcellulose (11%) + crystalline cellulose (89%) (Avicel RC-591). The rats were also given the corresponding volume of vehicle by oral gavage in the control sessions. Suspensions were administered in a final volume of 1 mL/kg. The drugs were given 30 minutes apart in the interaction experiments. A 6-hour sleep recording was started at approximately 8:30 A.M., 20 minutes after vehicle or drug(s) administration.

A balanced order of drug and control administrations was used to merge the effects of both drug and time elapsed during the protocol. At least 5 days were allowed between experiments to avoid long-lasting and rebound effects on sleep.

Statistics

A one-way analysis of variance (ANOVA) with repeated measures was used for statistical comparison of three or more samples. The results showing significant overall changes were subjected to a Newman-Keuls test to identify the changes that differed significantly from the baseline values.

RESULTS

The effect of BP 2.94 on the amount of each behavioral state is shown in Table 1. Doses of 20 and 30 mg/kg (8.18 and 12.2 mg/kg of the active constituent, respectively) significantly increased SWS. Wakefulness, light sleep, REM sleep, and sleep latencies showed slight but inconsistent reductions. Slow wave sleep enhancement after the 30-mg/kg dose was apparent along the 6-hour recording period, the increase attaining significance during the second and sixth hour (Figure 1). In contrast, no significant differences in REM sleep values compared to control were observed after BP 2.94 administration as judged by the hour-by-hour analysis (Figure 2).

Table 2. Effects of Carboperamide (MR 16155) on Sleep and Waking

Treatment	W	LS	SWS	REMS	SWSL	REMSL
Control	90.2 ± 5.1	50.8 ± 7.3	178.4 ± 8.2	40.6 ± 3.0	23.1 ± 6.1	37.4 ± 6.6
Carboperamide						
3	91.1 ± 8.3	50.0 ± 5.6	176.0 ± 9.2	42.9 ± 5.1	15.6 ± 3.8	40.4 ± 7.8
10	112.2 ± 7.1	42.3 ± 4.9	176.9 ± 6.0	28.6 ± 3.2***	21.7 ± 4.7	55.1 ± 10.5
20	138.9 ± 12.5**	55.9 ± 6.4	140.0 ± 13.1**	25.2 ± 4.1***	35.0 ± 10.3	64.7 ± 13.7
30	146.5 ± 20.0***	43.7 ± 6.5	141.8 ± 16.5**	28.0 ± 3.9**	43.2 ± 9.4	69.0 ± 16.2

W = waking; LS = light sleep; SWS = slow wave sleep; REMS = REM sleep; SWSL = slow-wave sleep latency; REMSL = REM sleep latency. All values are the means ± SEM (minutes). Nine animals were in each experimental group. Doses are in mg/kg. Statistical comparison with control values: ** $p < .01$; *** $p < .005$ (Newman-Keuls test).

Table 3. Effects of Pretreatment with Carboperamide on the BP 2.94-Induced Changes of Sleep and Waking

Treatment	W	LS	SWS	REMS	SWSL	REMSL
Control	90.2 ± 5.1	50.8 ± 7.3	178.4 ± 8.2	40.6 ± 3.0	21.1 ± 6.1	37.4 ± 6.6
BP 2.94 30	83.2 ± 7.0	37.8 ± 5.9	205.4 ± 6.9**	33.6 ± 2.8**	16.9 ± 4.4	47.0 ± 7.3
Carboperamide 30	146.6 ± 20.0***	43.7 ± 6.5	141.7 ± 16.5*	28.0 ± 3.9***	43.2 ± 9.4	68.9 ± 16.2
Carboperamide 30 + BP 2.94 30	118.7 ± 14.1	42.0 ± 5.1	174.6 ± 15.6	24.7 ± 4.1***	21.2 ± 5.7	69.1 ± 15.8

W = waking; LS = light sleep; SWS = slow wave sleep; REMS = REM sleep; SWSL = slow-wave sleep latency; REMSL = REM sleep latency. All values are the means ± SEM (minutes). Nine animals were in each experimental group. Doses are in mg/kg. Statistical comparison with control values: * $p < .02$ ** $p < .01$; *** $p < .005$ (Newman-Keuls test).

As shown in Table 2, carboperamide, 20 to 30 mg/kg, produced a significant and dose-dependent increase of waking, whereas SWS was reduced during the 6-hour recording period. After administration of 10 to 30 mg/kg of carboperamide REM sleep was significantly suppressed.

Carboperamide (30 mg/kg) prevented the increase in SWS produced by the 30-mg/kg dose of BP 2.94. The reduction of REM sleep described after administration of carboperamide alone was still present in rats given the combined treatment (Table 3).

DISCUSSION

The major finding of the present study is that the H_3 receptor agonist BP 2.94 increases SWS without suppressing REM sleep. On the other hand, the H_3 receptor antagonist carboperamide (MR 16155) increases waking and reduces SWS and REM sleep. The effect of BP 2.94 on SWS was prevented by prior administration of carboperamide.

Effects on sleep and waking after oral administration of prodrug BP 2.94 differ from those observed following direct injection of (R)-α-methylhistamine into the premammillary area of the rat. Thus, the latter induces an increase of SWS but suppresses REM sleep (Monti et al. 1991).

It could be argued that changes in sleep variables after oral administration of BP 2.94 are related to discomfort or malaise following the activation of the H_3 receptor seen in some peripheral tissues (e.g., lung, gastrointestinal tract, vascular smooth muscle, skin; van der Werf and Timmerman 1989; Schwartz et al. 1990). However, intraperitoneally administered (R)-α-methylhistamine fails to modify sleep/waking stages in the rat (Monti et al. 1991), which tends to indicate that the BP 2.94-mediated enhancement of SWS could be tentatively related to activation of H_3 receptors localized within the CNS. On the other hand, increased amounts of wakefulness after carboperamide administration could depend on the blockade of central H_3 receptors.

However, it should be also considered that histamine H_3 heteroreceptors have been characterized in central catecholamine, indolamine, and acetylcholine nerve endings. In vitro studies have shown that activation of these receptors with either histamine or (R)-α-methylhistamine inhibits the release of noradrenaline and serotonin from the cerebral cortex; acetylcholine from the entorhinal cortex, and dopamine from the striatum of rats and/or mice. This effect is prevented by the H_3 receptor antagonist thioperamide (Schlicker et al. 1988, 1989, 1993; Clapham and Kilpatrick 1992). Because a good deal of evidence favors a role for noradrenaline, dopamine, serotonin, and acetylcholine in the control of the waking EEG (Monti et al. 1988; Gaillard 1985; Dugovic 1992), it could be suggested that both the decreased availability of histamine at H_1 receptors located in the cerebral cortex, limbic system, corpus striatum, and hypothalamus and the inhibition of monoamines and acetylcholine release at critical areas in the CNS are responsible for SWS increase after BP 2.94 administration. However, further studies are needed to resolve this issue.

In conclusion, the histamine H_3 receptor agonist BP 2.94 shows the ability to increase SWS in rats, whereas carboperamide, which behaves as an H_3 receptor antagonist, increases waking and decreases SWS and REM sleep.

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