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The 5-HT_{1A} Antagonist (-)-Alprenolol Fails to Modify Sleep or Zimeldine-Induced Sleep–Waking Effects in Rats

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BJORVATN, B., D. NECKELMANN AND R. URSIN. *The 5-HT_{1A} antagonist (-)-alprenolol fails to modify sleep or zimeldine-induced sleep–waking effects in rats.* PHARMACOL BIOCHEM BEHAV **42**(1) 49–56, 1992.—Sleep and waking in rats were studied for 8 h following administration of a selective 5-hydroxytryptamine (5-HT) reuptake inhibitor (zimeldine), a putative 5-HT_{1A} antagonist {L(-)-alprenolol hydrogene tartrate monohydrate [(-)-alprenolol]} and a combination of (-)-alprenolol and zimeldine. Consistent with earlier findings, zimeldine gave a biphasic effect on sleep and waking. Waking was increased during the first 3 h, followed by a small decrease. Deep slow-wave sleep (SWS-2) showed the opposite trend. An initial decrease in SWS-2 was followed by an increase after around 3 h. Rapid eye movement sleep was markedly suppressed and latencies to sleep increased after zimeldine. (-)-Alprenolol had no effects on the different sleep and waking stages or latencies to sleep. The 5-HT_{1A} antagonist also failed to modify the effects of zimeldine administration. The behavioral syndrome induced by a selective 5-HT_{1A} agonist [8-hydroxy-2-(di-*n*-propyl-amino)-tetralin (8-OH-DPAT)] was clearly antagonized by administration of (-)-alprenolol, indicating that (-)-alprenolol was an efficient 5-HT_{1A} blocker. The data indicate that the sleep–waking effects of zimeldine cannot easily be explained by stimulation of 5-HT_{1A} receptors.

Waking Slow-wave sleep Rapid eye movement sleep Serotonin Zimeldine (-)-Alprenolol 5-HT reuptake inhibition 5-HT_{1A} blockade

SLEEP after administration of drugs that affect the serotonergic system has been extensively investigated. Still, the role of serotonin (5-HT) in the sleep-waking cycle is unclear. Several different 5-HT receptors are now known to exist in the brain: 5-HT_1, 5-HT_2, 5-HT_3, and 5-HT_4 receptors (7,15,32, 33). The 5-HT_1 receptor has further been divided into several subtypes: 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, and 5-HT_{1E} (15, 18,32). The effects on sleep and waking after selectively manipulating the individual receptors are likely to improve the understanding of the functional role of 5-HT in the sleep/waking cycle.

5-HT reuptake inhibitors are thought to increase available serotonin in the synaptic gap. Zimeldine, a selective 5-HT reuptake inhibitor, produced a double or biphasic effect on sleep and waking in rats (5,47). An initial increase in waking was followed by an increase in deep slow-wave sleep (SWS-2) after around 3 h. 5-HT_2 receptor agonists have been shown to increase waking (11) while 5-HT_2 antagonists enhance SWS-2 (10,11,23,24). In a recent study (5), the role of 5-HT_2 receptors on the effects of zimeldine was investigated. Pretreatment with ritanserin, a selective 5-HT_2 antagonist, did not block the initial waking effect seen after zimeldine administration. This indicates that the initial waking effect of zimeldine cannot be explained by 5-HT_2 stimulation. The waking effect then may be due to stimulation of other receptor subtypes.

5-HT_{1A} receptor agonists have also shown to increase waking (1,12). The effect of 5-HT_{1A} antagonists on sleep and waking stages are obscure (43,50), partly because no antagonists have been sufficiently selective on these receptors. The compound L(-)-alprenolol hydrogene tartrate monohydrate [(-)-alprenolol] has recently been introduced as a 5-HT_{1A} antagonist (31,42). If the initial waking effect seen after zimeldine administration is due to 5-HT_{1A} stimulation, then pretreatment with (-)-alprenolol should block this effect. The present study was therefore designed to investigate the role of 5-HT_{1A} receptors on the sleep and waking effects seen after zimeldine administration. In Experiment 1a, rats received 4 mg/kg (-)-alprenolol. After evaluation of the results, another experiment (Experiment 1b) was carried out in a few animals employing a higher dose (8 mg/kg) of (-)-alprenolol.

5-HT_{1A} receptor agonists also induce a behavioral syndrome (4,22,25,38,45,46). In this study, the antagonizing effect of (-)-alprenolol on the 5-HT_{1A} receptors was investigated by evaluating its ability to reduce this behavioral syndrome induced by the 5-HT_{1A} agonist, 8-hydroxy-2-(di-*n*-propyl-amino)-tetralin (8-OH-DPAT) (Experiment 2).

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METHOD

Experiment 1a

Preparation. Eight male Møll-Wistar rats (Møllegaard, Denmark) weighing 235–285 g at implantation were used in the experiments. Rats were kept on a 12 L : 12 D schedule with lights on at 06:00. A mixture of Hypnorm Janssen-Dormicum Roche diluted with distilled water was used as anaesthesia (the mixture contained fentanyl, 0.05 mg/ml; fluanizone, 2.5 mg/ml; and midazolam 1.25 mg/ml). Rats were injected SC with 0.60 ml and implanted with stainless steel screw electrodes for frontofrontal and frontoparietal electroencephalogram (EEG) recording and silver wires in the neck muscle for electromyogram (EMG) recording. At least 2 weeks were allowed for recovery and adaptation prior to the start of recording.

Recording. Rats were placed individually inside 60-cm high clear plastic cylinders placed in plastic bins. The flexible recording cable and rotating connector hang in a movable arm just above the cylinder, permitting rats to move around unrestrained. Rats had free access to food pellets and water. Recording started right after the second injection around 0830 and lasted 8 h. No less than 7 days separated the different experiments.

Experimental design. Each rat received four different drug combinations: saline (0.9% NaCl) + saline, saline + zimeldine, (-)-alprenolol + saline, and (-)-alprenolol + zimeldine in a balanced-order blind design. The two drug injections were given with 1/2-h interval. All injections were administered IP.

Drugs. Zimeldine (36) was obtained from Astra Läkemedel AB, Södertälje, Sweden. It was injected IP in a dose of 20 mg/kg dissolved in saline. This dose was chosen to obtain a clear biphasic effect on sleep and waking as reported in an earlier study (47).

(-)-Alprenolol was obtained from Hässle AB, Sweden. It was injected IP in a dose of 4 mg/kg dissolved in saline.

Injected volumes were 0.50–0.65 ml.

Sleep scoring. EEG and EMG were recorded on a Grass polygraph. Frontoparietal EEG and EMG were also digitized on-line and stored on magnetic tape for further calculations. Scoring of the sleep and waking stages was obtained by an automatic system based on EEG power spectrum analysis of delta (1–4 c/s) and theta (6–9 c/s) activity. Scoring was based on the criteria given by Ursin and Larsen (48). Threshold values for the different vigilance states were determined by thoroughly comparing the polygraph record with power spectrum data in the saline + saline condition. Delta activity was used to discriminate between waking, SWS-1, and SWS-2. Rapid eye movement (REM) threshold was determined using the theta/delta ratio as a criterion (49). Total slow-wave sleep (SWS-1 + SWS-2) was also computed, as well as latencies to each sleep stage and to stable sleep. Stable sleep onset was defined as the first sleep episode lasting > 5 min and not being interrupted by a waking episode of > 1/2 min (44).

Statistics. Data were analyzed with a three-way analysis of variance (ANOVA). First injection [saline or (-)-alprenolol] served as factor 1, second injection (saline or zimeldine) as factor 2, and time (h 1–8) as factor 3. Data from the total 8-h period and sleep latency data were analyzed with a two-way ANOVA for repeated measures. Saline and (-)-alprenolol (first injection) as trials. Significant overall effects were evaluated with posthoc *t*-tests for dependent measures. All significance levels reported are two-tailed.

One of the rats lost its electrodes before the last experiment with saline + zimeldine, and in another rat the experiment (-)-alprenolol + saline was discarded because of computer failure. One of the (-)-alprenolol + zimeldine experiments was discarded due to problems with the drug injection. The missing data for these experiments were substituted with the means obtained for the remaining rats in the appropriate experiments.

Experiment 1b

In a separate experiment, three rats prepared as in Experiment 1a received 8 mg/kg (-)-alprenolol in addition to the other drug combinations. These rats received six different drug combinations: saline + saline, saline + zimeldine, (-)-alprenolol 4 mg/kg + saline, (-)-alprenolol 4 mg/kg + zimeldine, (-)-alprenolol 8 mg/kg + saline, and (-)-alprenolol 8 mg/kg + zimeldine.

Due to an update of equipment in the laboratory, rats were subjected to slightly different recording conditions. Rats in Experiment 1b were recorded in their home transparent plastic cages (minus the steel bar top) placed inside sound-attenuating chambers with a clear Plexiglas front door. Otherwise, recording conditions were similar to those of Experiment 1a. Also, sleep scoring and statistics procedures were the same.

Experiment 2

Preparation. Eight male Møll-Wistar rats following the same preparation as rats in Experiment 1 were used in the experiment.

Drugs. 8-OH-DPAT was obtained from Research Biochemicals, Inc. (Waltham, MA).

TABLE 1

SLEEP STAGES IN PERCENTAGE OF RECORDING TIME IN EXPERIMENT 1a

Sleep Stage	Control	Zimeldine	Alprenolol	Alprenolol + Zimeldine
Waking	53.9 ± 3.1	61.9 ± 4.1*	53.5 ± 3.6	61.3 ± 2.9‡
SWS-1	34.8 ± 2.7	29.0 ± 3.4†‡	36.7 ± 2.2	29.1 ± 2.0§
SWS-2	6.7 ± 1.3	8.9 ± 2.3	5.8 ± 1.6	9.7 ± 2.7
T SWS	41.5 ± 3.3	37.9 ± 4.2	42.5 ± 3.6	38.8 ± 2.9
REM sleep	4.7 ± 0.6	0.2 ± 0.1†¶	4.1 ± 0.6	0.02 ± 0.01†¶

Values are means ± SEM. Symbols indicate significance levels of posthoc *t*-tests.
*$p < 0.01$, †$p < 0.001$, compared with the control (NaCl/NaCl) condition.
‡$p < 0.05$, §$p < 0.01$, ¶$p < 0.001$, compared with the (-)-alprenolol/NaCl condition.

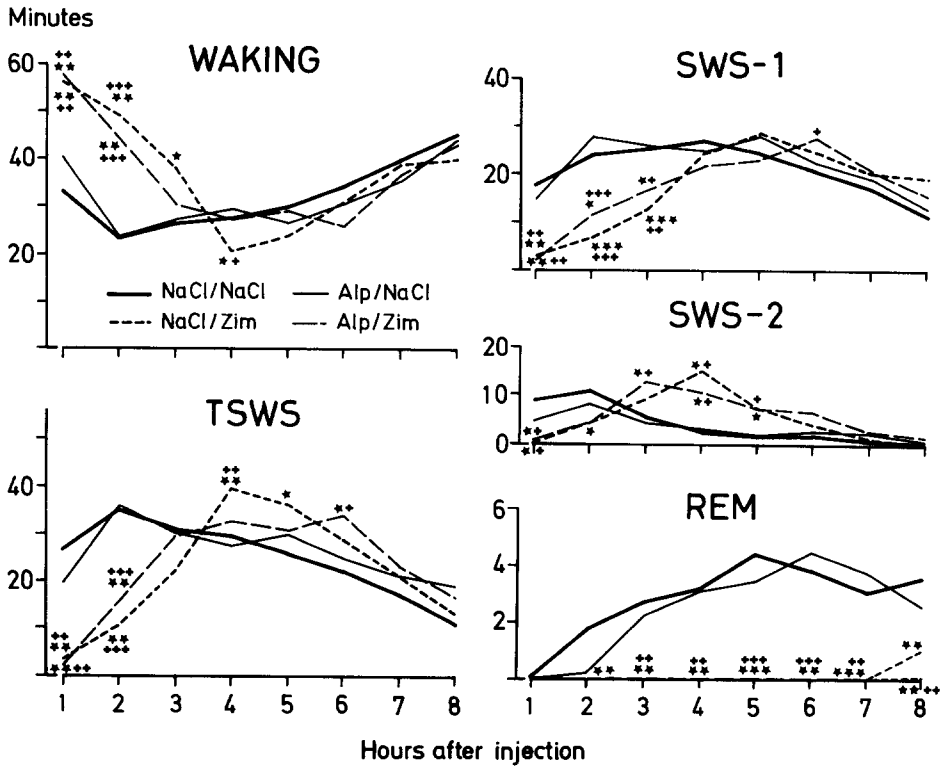

FIG. 1. Minutes per hour recording period spent in the different stages following NaCl + NaCl (thick line), NaCl + zimeldine (dotted line), (-)-alprenolol + NaCl (thin line), and (-)-alprenolol + zimeldine (broken line) conditions. *,**,***Significantly different from control condition; +,++,+++ Significantly different from (-)alprenolol + NaCl condition; $p < 0.05$, 0.01, and 0.001, respectively.

Recording and design. Rats were placed individually in transparent plastic cages inside sound-attenuating chambers with a clear Plexiglas front door. Rats were adapted for at least 14 days. Drugs were given in a balanced-order design. At least 5 days separated the two test situations. Thirty minutes before videotape recording, rats received IP injection of either 0.50 ml saline or 4.0 mg/kg (-)-alprenolol dissolved in 0.50 ml saline. Immediately before recording, rats received 0.50 mg/kg 8-OH-DPAT dissolved in 0.50 ml saline SC. Rats were then videotaped for the consecutive 30 min.

Based on the videotapes, the following signs were rated in the individual rat by a rater blind to the treatment given: a) forepaw-treading, b) hindlimb abduction, c) flattened body posture, d) hyperlocomotion, and e) head-weaving. The observation period consisted of three consecutive 15-s periods, repeated every 5 min, the first period 5 min after the last injection. A four-point ranked intensity scale was used (0 = absent, 1 = equivocal, 2 = definite, 3 = intense) (17,38). The median scores of the three consecutive 15-s periods were used as the score from each 5 min period.

Statistics. Rated scores were analyzed with ANOVA, first in a three-way design (category of behavior, treatment, and

TABLE 2

LATENCIES TO EACH SLEEP STAGE AND TO STABLE SLEEP IN MINUTES FOLLOWING INJECTIONS IN EXPERIMENT 1a

Sleep Stage	Control	Zimeldine	Alprenolol	Alprenolol + Zimeldine
SWS-1	8.7 ± 4.2	44.2 ± 15.3	14.5 ± 6.9	36.3 ± 9.2*
SWS-2	25.6 ± 6.5	111.2 ± 15.2†‡	38.0 ± 9.9	109.4 ± 15.8†§
REM sleep	95.4 ± 12.6	466.1 ± 6.0†§	126.5 ± 21.5	474.6 ± 4.7†§
Stable sleep	37.2 ± 11.3	148.4 ± 16.7†§	47.1 ± 10.2	122.4 ± 14.8†§

Values are means ± SEM. Symbols indicate significance levels of posthoc *t*-tests.
*$p < 0.05$, †$p < 0.001$, compared with the control (NaCl/NaCl) condition.
‡$p < 0.01$, §$p < 0.001$, compared with the (-)-alprenolol/NaCl condition.

time), then in a two-way design (treatment and time). When ANOVA showed significant changes of pretreatment with (-)-alprenolol, Wilcoxon matched-pairs tests with one-tailed probability were performed at each time point.

RESULTS

Experiment 1a

Behavioral effects. No obvious behavioral changes were seen after any of the drug combinations.

Effects on sleep and waking. The total amounts of sleep and waking over the whole recording period are shown in Table 1. The hourly distribution of the different stages are shown in Fig. 1. Latencies to the different sleep stages and to stable sleep are shown in Table 2. It appeared that (-)-alprenolol had no effects on the different sleep-waking stages or on latencies to sleep. Zimeldine, on the other hand, showed clear effects on all sleep and waking stages and latencies. The combination of (-)-alprenolol + zimeldine had similar effects as the zimeldine-alone condition.

Waking. There was no effect of (-)-alprenolol on the waking stage, neither in the total 8-h period (Table 1) nor in the hourly distribution [Fig. 1, three-way ANOVA: $F(1, 7) = 0.03$, $p = 0.85$].

Zimeldine increased waking in the total 8-h period [two-way ANOVA: $F(1, 7) = 26.34$, $p < 0.005$]. Posthoc t-test showed the most pronounced increase was seen after the zimeldine alone condition (Table 1).

A study of the hourly distribution of waking (Fig. 1) revealed that the increase in total waking was caused by an increase in the first 3 h of recording. Three-way ANOVA showed a clear effect in waking of zimeldine $F(1, 7) = 26.59$, $p < 0.005$, and also an interaction between zimeldine and time, $F(7, 49) = 12.60$, $p < 0.00001$, indicating variation in the treatment effect over time. In the fourth hour, a small decrease in waking was recorded after zimeldine alone (Fig. 1).

The waking effect induced by zimeldine was not modified after (-)-alprenolol administration (Fig. 1).

SWS-1. There was no effect of (-)-alprenolol on total SWS-1 (Table 1) or the hourly distribution of SWS-1 [Fig. 1, three-way ANOVA: $F(1, 7) = 0.12$, $p = 0.73$].

Zimeldine decreased SWS-1 in total 8-h period [Table 1, two-way ANOVA: $F(1, 7) = 39.48$, $p < 0.001$]. The decrease in SWS-1 was seen only during the first 3 h (Fig. 1). From hour 4, SWS-1 returned to control values. Three-way ANOVA on the hourly distribution indicated a clear SWS-1 effect of zimeldine, $F(1, 7) = 39.32$, $p < 0.001$, and once again treatment × time interaction, $F(7, 49) = 12.96$, $p < 0.00001$. The reduction of SWS-1 induced by zimeldine was not modified after (-)-alprenolol administration.

SWS-2. Again, (-)-alprenolol had no effect [Table 1; Fig. 1, three-way ANOVA: $F(1, 7) = 0.0007$, $p = 0.93$].

Zimeldine gave a small overall increase in SWS-2 in the total 8-h period [Table 1, two-way ANOVA: $F(1, 7) = 5.79$, $p < 0.05$].

Zimeldine alone and the combination (-)-alprenolol + zimeldine had a clear biphasic effect on the hourly distribution of the SWS-2 stage [Fig. 1, three-way ANOVA: $F(7, 49) = 9.77$, $p < 0.00001$]. First, a decrease in SWS-2 was seen and then from hour 3–5 an increase was recorded. After the fifth hour, SWS-2 returned to control values. The effect of zimeldine on SWS-2 was not modified after (-)-alprenolol administration (Fig. 1).

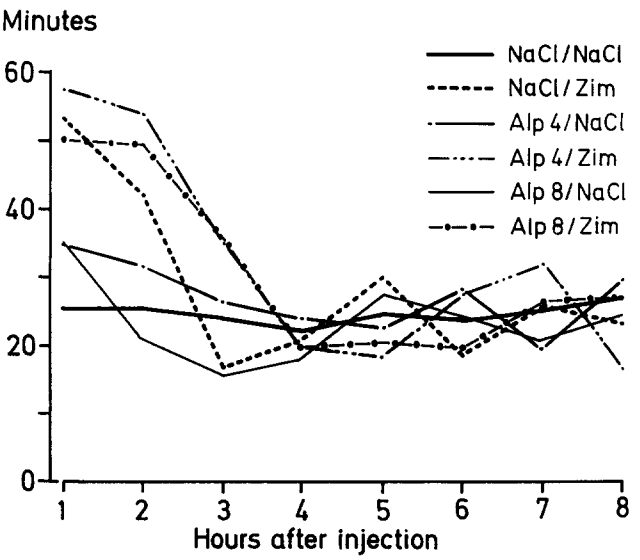

FIG. 2. Minutes per hour recording period spent in the waking stage following NaCl + NaCl, NaCl + zimeldine, 4 mg/kg (-)-alprenolol + NaCl, 4 mg/kg (-)-alprenolol + zimeldine, 8 mg/kg (-)-alprenolol + NaCl, and 8 mg/kg (-)-alprenolol + zimeldine conditions.

Total slow-wave sleep (TSWS). There was no effect of (-)-alprenolol on total TSWS (Table 1) or the hourly distribution of TSWS [Fig. 1, three-way ANOVA: $F(1, 7) = 0.08$, $p = 0.77$].

A small decrease was seen after zimeldine on total TSWS [Table 1, two-way ANOVA: $F(1, 7) = 6.76$, $p < 0.05$]. As expected from the SWS-1 and SWS-2 data, a biphasic effect was seen in the hourly distribution after zimeldine alone and in combination with (-)-alprenolol (Fig. 1). Three-way ANOVA showed as expected a weak overall effect of zimeldine, $F(1, 7) = 6.68$, $p < 0.05$, and a clear interaction with time, $F(7, 49) = 15.79$, $p < 0.00001$.

REM sleep. There was no effect of (-)-alprenolol on total REM sleep (Table 1) or the hourly distribution of REM [Fig. 1, three-way ANOVA: $F(1, 7) = 1.12$, $p = 0.33$].

REM sleep showed a marked reduction in the total 8-h period after zimeldine administration [Table 1, two-way ANOVA: $F(1, 7) = 82.16$, $p < 0.0005$].

Figure 1 gives the results on an hour-to-hour basis. REM sleep was markedly decreased after zimeldine from hour 2 [Fig. 1, three-way ANOVA: $F(1, 7) = 82.03$, $p < 0.0005$] and a noticeable treatment × time interaction was seen, $F(7,49) = 13.50$, $p < 0.00001$. Posthoc t-tests showed this reduction in REM sleep was similar for the zimeldine alone and the (-)-alprenolol + zimeldine conditions except in hour 8 (Fig. 1).

Sleep latencies. There was no effect of (-)-alprenolol on the latencies to SWS-1, SWS-2, REM, or stable sleep [Table 2, two-way ANOVA: $F(1, 7) = 0.02, 0.26, 1.78, 0.64$, $p = 0.85, 0.63, 0.22, 0.45$, respectively].

Zimeldine increased latency to SWS-1, SWS-2, REM, and stable sleep [Table 2, two-way ANOVA: $F(1, 7) = 6.84, 96.72, 880.95, 74.32$, $p < 0.05, 0.0005, 0.00001, 0.0005$, respectively]. Posthoc t-tests indicated the increases were seen after both zimeldine alone and the (-)-alprenolol + zimeldine combination for SWS-2, REM, and stable sleep (Table 2). In SWS-1, only the combination gave a significant increase

TABLE 3

SLEEP STAGES IN PERCENTAGE OF RECORDING TIME IN EXPERIMENT 1b

Sleep Stage	Control	Zimeldine	Alp4	Alp4 + Zim	Alp8	Alp8 + Zim
Waking	41.4 ± 7.6	48.4 ± 6.4	45.5 ± 7.7	54.8 ± 4.1	39.5 ± 4.5	52.1 ± 8.0
SWS-1	33.8 ± 6.3	34.4 ± 2.8	29.1 ± 5.3	24.7 ± 5.1	39.3 ± 3.2	29.1 ± 3.3
SWS-2	18.8 ± 6.2	17.2 ± 4.8	20.6 ± 8.0	20.4 ± 6.6	17.1 ± 6.1	18.8 ± 6.5
T SWS	52.5 ± 5.9	51.5 ± 6.4	49.7 ± 6.6	45.1 ± 4.0	56.4 ± 3.3	47.9 ± 8.0
REM sleep	6.0 ± 1.8	0.0 ± 0.0	4.8 ± 1.1	0.1 ± 0.1	4.2 ± 1.2	0.0 ± 0.0

Values are means ± SEM. Alp4, (-)-alprenolol 4 mg/kg; Alp8, (-)-alprenolol 8 mg/kg; Zim, zimeldine.

compared to control condition. Zimeldine alone had a t-test value of 2.22 ($p = 0.061$).

Experiment 1b

This experiment was performed after the results from Experiment 1a showed no effect of 4 mg/kg (-)-alprenolol.

In particular, it was of interest to check if the 8-mg/kg dose of (-)-alprenolol would diminish the initial waking effect seen after zimeldine administration. No such effect on waking was seen [Fig. 2, three-way ANOVA: $F(2, 4) = 1.10$, $p = 0.42$]. The three rats showed the same effects of zimeldine and lack of effects of (-)-alprenolol on sleep and waking.

Since these three rats were recorded at a later date and under slightly different conditions (see the Method section), we decided not to include rats from Experiment 1b in 1a. However, the sleep-waking data were within two standard deviations from Experiment 1a, except for the SWS-2 stage. The total amounts of sleep and waking over the whole recording period are shown in Table 3.

Experiment 2

8-OH-DPAT induced a clear 5-HT behavioral syndrome (Fig. 3). Pretreatment with 4.0 mg/kg alprenolol IP showed significant effect on the rated behaviors [three-way ANOVA: $F(1, 7) = 21.33$, $p < 0.005$]. Total score was significantly decreased in all rating periods except for the first (Wilcoxon matched pair test, one tailed, Fig. 3).

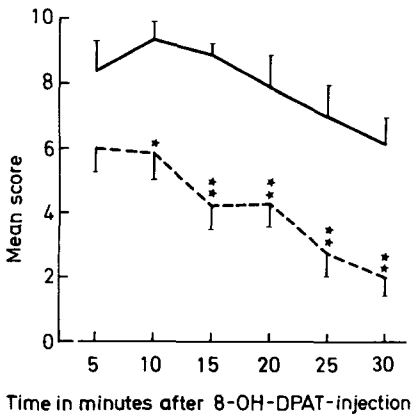

FIG. 3. Time course of total behavioral score (all rated components) ± SEM in rats pretreated with NaCl (unbroken line) or with (-)-alprenolol (broken line).*,**Significantly different from NaCl condition; $p < 0.05$, 0.01, respectively.

Two-way ANOVA showed significant effects on the rated categories hindlimb abduction, $F(1, 7) = 28.06$, $p < 0.005$, flattened body posture, $F(1, 7) = 15.40$, $p < 0.01$, hyperlocomotion, $F(1, 7) = 22.67$, $p < 0.01$, and head-weaving, $F(1, 7) = 8.59$, $p < 0.05$, but not on forepaw-treading, $F(1, 7) = 2.85$ (Fig. 4).

Wilcoxon matched-pairs tests (one tailed) were performed on each time point in the categories of behavior where ANOVA showed significant changes (Table 4).

DISCUSSION

Zimeldine had a biphasic effect on sleep and waking stages. Waking was increased during the first 3 h, followed by a decrease. Deep slow-wave sleep (SWS-2) showed the opposite trend, first being significantly decreased and after 3–4 h increasing. These results are in agreement with other studies in our laboratory (5,47). The effects of zimeldine on SWS-1, REM, and latencies to sleep and stable sleep are also in accordance with earlier studies. However, for no obvious reason rats showed less SWS-2 than in the former study (5). The sleep-waking effects of zimeldine are considered serotonergic (5,34,39,47).

Administration of (-)-alprenolol had no effect on the dif-

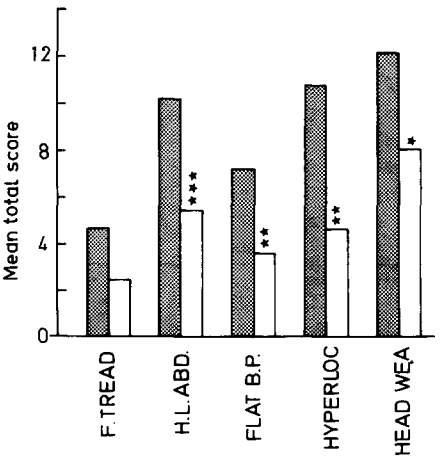

FIG. 4. Summated scores for all 5-min rating points for each of the components—forepaw-treading (F. Tread), hind limb abduction (H. L. Abd.), flattened body posture (Flat. B. P.), hyperlocomotion (Hyperloc.), head-weaving (Head wea.)—in rats pretreated with NaCl (filled bars) or with (-)-alprenolol (open bars). *,**,***Significantly different from NaCl condition; $p < 0.05$, 0.01, and 0.001, respectively.

TABLE 4
SCORE FOR EACH BEHAVIORAL CATEGORY FOR EACH RATING PERIOD

Time	Group	Forepaw-Treading	Hindlimb Abduction	Flattened Body Posture	Hyperlocomotion	Head-Weaving
5	NaCl	0.50 ± 0.27	2.0 ± 0.27	1.4 ± 0.36	2.3 ± 0.16	2.3 ± 0.16
	Alp	0.50 ± 0.19	1.1 ± 0.30*	0.88 ± 0.35	1.6 ± 0.26*	1.9 ± 0.13
10	NaCl	0.88 ± 0.30	2.3 ± 0.16	1.9 ± 0.23	2.1 ± 0.13	2.3 ± 0.16
	Alp	0.63 ± 0.26	1.3 ± 0.31*	0.63 ± 0.18*	1.3 ± 0.31*	1.9 ± 0.30
15	NaCl	1.13 ± 0.30	2.0 ± 0.0	1.6 ± 0.26	2.0 ± 0.0	2.1 ± 0.13
	Alp	0.38 ± 0.18	0.75 ± 0.31*	0.13 ± 0.13*	1.3 ± 0.25*	1.1 ± 0.30*
20	NaCl	0.88 ± 0.23	1.5 ± 0.27	1.1 ± 0.30	1.9 ± 0.30	1.9 ± 0.13
	Alp	0.38 ± 0.26	0.75 ± 0.31*	0.25 ± 0.16*	1.1 ± 0.30	1.3 ± 0.37
25	NaCl	0.75 ± 0.31	1.3 ± 0.25	1.1 ± 0.30	1.6 ± 0.26	1.9 ± 0.13
	Alp	0.38 ± 0.18	0.13 ± 0.13*	0.25 ± 0.16*	0.63 ± 0.18*	1.0 ± 0.33*
30	NaCl	0.50 ± 0.27	1.1 ± 0.23	0.50 ± 0.19	1.5 ± 0.27	1.8 ± 0.16
	Alp	0.13 ± 0.13	0.0 ± 0.0*	0.0 ± 0.0*	0.38 ± 0.26*	0.9 ± 0.35*

Values are means ± SEM.
*Significance of posthoc Wilcoxon matched-pairs test ($p < 0.05$).

ferent sleep and waking stages. The drug also failed to modify the effects seen after zimeldine administration. It is reported that (-)-alprenolol has appreciable affinity for the 5-HT_{1A} receptor (31,42). Since administration of selective 5-HT_{1A} agonists increase waking (1,12), it could be anticipated that antagonist administration might decrease waking. The reason for this lack of effect on the sleep/waking cycle is not clear. However, there are several possible explanations.

First, (-)-alprenolol may not block 5-HT_{1A} receptors in the dose applied. To check for this possibility, Experiment 2 was carried out. 5-HT_{1A} agonists are well-known potent inducers of the 5-HT behavioral syndrome, a syndrome typically consisting of forepaw-treading, hindlimb abduction, flattened body posture, hyperlocomotion, and head-waving (4,22,25, 38,45,46). 5-HT_{1A} antagonists have been found to counteract this induction (25,30,31,38,45,46). We used the putative selective 5-HT_{1A} agonist 8-OH-DPAT, which in other studies has been found to induce the syndrome in a dose-related manner at doses of 0.03–2.5 mg/kg (38,45). Pretreatment with (-)-alprenolol clearly reduced signs of the behavioral syndrome. This finding indicated that 4 mg/kg (-)-alprenolol was an effective dose for antagonizing the 5-HT_{1A} receptors.

To check if a higher dose of (-)-alprenolol would affect sleep and waking or the zimeldine-induced sleep–waking changes, a separate experiment (Experiment 1b) was carried out with 8 mg/kg (-)-alprenolol. In particular, a possible waking reduction was evaluated. No sign of any sleep–waking effect was seen in the three rats. The dose of zimeldine used was within the dose range of other sleep studies (34) and in the lower dose range of a behavioral study by Altman et al. (3).

Second, the possibility that the effect of (-)-alprenolol on sleep and waking was too short lived to be detected must be considered. It took 1/2 h from the injection of (-)-alprenolol until recording started. The behavioral data indicated, however, that (-)-alprenolol had a clear antagonizing effect on 5-HT_{1A} receptors at least until the end of the videotape recording 1 h after injection (Fig. 3). Also, with a half-life of 80 min in rats (37), a possible sleep–waking effect would be expected to last at least for another 1/2 to 1 h.

A third possibility is that (-)-alprenolol exerts effects on other receptors or transmittor systems, thereby counteracting any sleep/waking effects on the 5-HT_{1A} receptor. β-Adrenoceptor blocking drugs, like (-)-alprenolol, are not selective on the 5-HT_{1A} receptor subtype but also act on 5-HT_{1B} receptors (2,13,30,31,41,42). 5-HT_{1B} receptors are shown to be located as presynaptic autoreceptors (13,32) and blocking these receptors with (-)-alprenolol may increase 5-HT release. This may then counteract the effects of (-)-alprenolol on the 5-HT_{1A} receptors. Also, the effect of (-)-alprenolol on β-adrenergic receptors may affect sleep and waking. Kalkman and Soar showed that the 5-HT_{1A} receptor antagonist effects of pindolol on the behavioral syndrome was masked by β-adrenoceptor blockade (25). One study showed that β-blockade reduced REM sleep in rats but had no effects on other stages (26). In cats, such β-adrenergic blockade decreased REM and deep SWS and increased drowsy waking (20). No REM-sleep-reducing effect of (-)-alprenolol was seen in the present study.

Also, the location of the different 5-HT receptors may be of importance. Several reports indicate that 5-HT_{1A} is a postsynaptic receptor (6,8,16,28). However, the 5-HT_{1A} subtype has also been shown to be located as a somatodendritic autoreceptor in the dorsal raphe nuclei (9,28,41). In addition to blocking postsynaptic receptors, (-)-alprenolol may have blocked the autoreceptor (30) and therefore caused increased firing of the neurons. These two effects of (-)-alprenolol may have counteracted each other on any possible effect on sleep and waking. It has recently been suggested that increased locomotor activity and increased incidence of flat body posture following 8-OH-DPAT injection may be caused by stimulation of the somatodendritic 5-HT_{1A} receptors (19). In the present study, both hyperlocomotion and the incidence of flat body posture were antagonized by (-)-alprenolol, suggesting a 5-HT_{1A} autoreceptor effect of this drug.

Studies with other putative 5-HT_{1A} antagonists are few and conflicting. One study showed that (-)-propranolol injected intraperitoneally increased waking, decreased synchronized sleep, and abolished REM sleep (50); another showed decreased waking and increased REM sleep after infusion into the dorsal pontine tegmentum (43). The reason why this 5-HT_{1A} antagonist and β-adrenoceptor blocker affected the sleep/waking stages while (-)-alprenolol did not is not clear.

The initial waking effect seen after zimeldine administration, then, cannot easily be explained by 5-HT_{1A} stimulation

considering the results in this study. There was no sign of (-)-alprenolol reducing the zimeldine-induced waking effect. Data from a previous study (5) indicated the waking effect was not reduced by a 5-HT_2 antagonist either. There are other receptors that might be involved. 5-HT_{1B} receptors may be located postsynaptically in addition to the location as a terminal autoreceptor (14,28). The lack of selective antagonists for the 5-HT_1 subtypes makes it difficult to test if the waking effect of zimeldine is caused by stimulation of these receptors.

It is possible, however, that the initial waking period seen after zimeldine was caused by some other serotonergic effect incompatible with sleep. Behavioral and motor phenomena were seen after 5-HT_1 stimulation in this and other studies (17,21). The phenomena observed after 8-OH-DPAT administration in our study were hardly compatible with sleep. The behavioral syndrome was reduced but not all components were antagonized by (-)-alprenolol. In this study, however, no obvious behavioral changes of zimeldine were seen. In cats, serotonin reuptake inhibitors induced behavioral and motor changes (40). Other serotonergic effects possibly interfering with sleep could be alternations in temperature control (51), in vegetative functioning (29,39) or in sensory modulation (35).

There was no sign of (-)-alprenolol altering the delayed increase in SWS-2 seen 3–4 h after zimeldine administration. Considering that (-)-alprenolol has a half-life in rats of 80 min (37), it cannot be concluded from this study whether (-)-alprenolol has any effect on the zimeldine-induced SWS-2 increase.

The biphasic effect of zimeldine may also be caused by receptor kinetic effects. The delayed sleep effect may result from a shutoff of neuron firing and transmittor release because of autoreceptor stimulation, resulting in diminished postsynaptic waking effect. Leysen et al. (27) showed that 5-HT_2 receptors desensitized within a few hours after injections of a 5-HT_2 receptor agonist. Administration of zimeldine may also possibly desensitize receptors and thereby cause the biphasic effect seen on sleep and waking.

In conclusion, this study showed that the initial waking effect seen after administration of the serotonergic reuptake inhibitor, zimeldine, cannot easily be explained by 5-HT_{1A} stimulation. In fact, blocking 5-HT_{1A} receptors with systemically administered (-)-alprenolol gave no effects on sleep and waking.

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