Pharmacology Biochemistry and Behavior, Vol. 44, pp. 797–804, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Effects of Chronic Treatment With Two Selective 5-HT_2 Antagonists on Sleep in the Rat

ROSS H. PASTEL,*[1] EDWARD ECHEVARRIA,* BARRY COX,†
THOMAS P. BLACKBURN‡ AND FRANK C. TORTELLA*

**Department of Medical Neurosciences, Division of Neuropsychiatry, Walter Reed Army Institute of Research, Washington, DC 20307*
†ICI Pharmaceuticals Division, Bioscience II, Alderly Park, Macclesfield, Cheshire SK10 4TG, UK
‡SmithKline Beecham Pharmaceuticals, Coldharbour Road, The Pinnacles, Harlow, Essex, CM19 5AD, UK

Received 11 May 1992

PASTEL, R. H., E. ECHEVARRIA, B. COX, T. P. BLACKBURN AND F. C. TORTELLA. *Effects of chronic treatment with two selective 5-HT_2 antagonists on sleep in the rat.* PHARMACOL BIOCHEM BEHAV 44(4) 797–804, 1993.—The effect of chronic administration of 2(2-dimethylaminoethylthio)-3-phenylquinoline (ICI-169,369) and 2(2-dimethylamino-2-methylpropylthio)-3-phenylquinoline (ICI-170,809), two selective 5-HT_2 antagonists, on sleep was studied in rats. As previously shown, the acute effect of ICI-170,809 was to increase latency to rapid eye movement sleep (REMS), decrease the number of REM periods (REMPs), suppress the cumulative amount of REMS over 12 h, and increase the duration of REMPs in the first 6 h, while having no effect on non-REM sleep (NREMS). Administration of ICI-169,369 had similar effects except no change was seen in the duration of REMPs and cumulative REMS was suppressed for 24 h. When given 2× daily for 5 days, tolerance to the REMS suppressant effects developed in both drugs. After discontinuation of treatment, a REMS rebound occurred after ICI-170,809, but not ICI-169,369. No significant effect on NREMS was seen after administration of ICI-170,809, whereas ICI-169,369 lowered 24-h cumulative NREMS on the fifth day of administration.

Serotonin$_2$ antagonists ICI-170,809 ICI-169,369 Sleep REM Rats

ALTHOUGH serotonin [5-hydroxytryptamine (5-HT)] has long been postulated to be involved in the regulation of the sleep–wakefulness cycle, its role has been subject to much debate (10,16). In the past, there were few specific drugs available to study the role of 5-HT in the control of sleep. In recent years, multiple 5-HT receptors and receptor subtypes have been discovered (26). Along with this discovery has been the development of more specific and selective 5-HT agents.

Recently, two chemically novel 5-HT_2 antagonists, 2(2-dimethylaminoethylthio)-3-phenylquinoline (ICI-169,369) and 2(2-dimethylamino-2-methylpropylthio)-3-phenylquinoline (ICI-170,809), have been described (3,15). Both have high affinity for the 5-HT_2 binding site in the rat cortex (K_i 1.79×10^{-8} M for ICI-169,369 and 6.6×10^{-10} M for ICI-170,809) while possessing relatively low affinity for other neurotransmitter receptors (3,8,15). In two in vivo tests of 5-HT_2 function, ICI-169,369 blocked 5-hydroxytryptophan (5-HTP)-induced head twitches and fenfluramine-induced hyperthermia (2). Both ICI-169,369 and ICI-170,809 have also been shown to block both 5-HTP and DOI (a 5-$HT_{2/1c}$ agonist)-induced head twitches and block 5-HT-evoked excitation of dorsal raphe neurons (8). Both compounds have good oral bioavailability [(2), Blackburn, personal communication]. Previously, we (38) described the acute effects of ICI-169,369 and ICI-170,809 on the 24-h sleep–wake cycle of rats. Both compounds induced a dose-related increase in the latency to rapid eye movement sleep (REMS) and suppressed cumulative REMS time. Non-REMS (NREMS) was not affected.

The purpose of the present experiments was to determine the effects of chronic administration of ICI-170,809 and ICI-169,369 on 24-h sleep patterns using a dose that produced effects in our acute study. We were especially interested in determining whether tolerance would develop to the REMS effects we observed after acute administration.

METHOD

Preparation of Animals

Male Sprague-Dawley rats (275–325 g) were implanted with cortical EEG and temporalis muscle EMG electrodes under

[1] To whom requests for reprints should be addressed.

Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NIH Publication 85-23. The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense (para 4-3), AR 360-5.

ketamine anesthesia (100–150 mg/kg, IP) (37). After surgery and throughout the experiment, rats were housed individually in full-view Plexiglas cages (27 × 30 × 50 cm) located in a shielded, sound-attenuated, temperature-controlled (25°C) chamber. Rats were maintained on a 12 L : 12 D cycle (light onset at 0600 h). Food and water were available ad lib throughout the study. Animals were allowed a minimum of 5 days to recover from surgery and acclimate to their new environment.

EEG and Behavior Analysis

EEG and EMG activity were monitored continuously on a 10-channel Grass polygraph (Grass Instruments, Quincy, MA). EEG was recorded at a speed of 30 mm/min and the records were manually scored in consecutive 10-min bins for total NREMS and REMS. Latency to NREMS was defined as the length of time from drug administration to the appearance of 3 consecutive min of high-amplitude, slow-wave EEG, whereas REMS latency was measured from the onset of NREMS to the first 15-s epoch of theta EEG with low EMG (REMS). Cumulative NREMS and REMS times were calculated for each 24-h period. Behavior was observed at 5-min intervals for 30 min following drug or vehicle administration. An initial discomfort/reaction characterized by several head shakes followed by face grooming after p.o. administration was observed, which lasted less than 10 min. After the initial reaction to drug administration, no abnormal behaviors were seen.

Drugs and Treatment Protocol

After normal diurnal sleep–wake activity was established (typically, 3–5 days), twice-daily oral administrations (p.o.) of control vehicle (distilled water) were given for 3 consecutive days. Treatments were given at 1000 and 1600 h. Eighteen hours following the last control treatment, 5 days of twice-daily ICI-170,809 (10 mg/kg, $n = 5$ animals) or ICI-169,369 (40 mg/kg, $n = 8$ animals) treatments ensued, followed by a 3-day recovery period during which sham treatments were given twice daily. The doses of the ICI compounds were selected based upon the results of our previously reported acute study (38). Sham treatments consisted of inserting the gavage needle into the mouth but not injecting any fluid. ICI-169,369 or ICI-170,809 were dissolved in distilled water and administered p.o. in a volume of 1 ml/kg. Using this protocol, each animal served as its own control. Animals were drug naive prior to study and were used for one study only.

Statistics

The days used for analysis were the third day of distilled water (VEH), the first (ICI-1) and fifth (ICI-5) days of drug administration, and the first (REC-1) and third (REC-3) days of recovery (i.e., sham treatment). Cumulative REMS and NREMS totals were analyzed using a two-factor analysis of variance (ANOVA) with two repeated measures (treatment × h). *A priori* planned comparisons were VEH vs. ICI-1, VEH vs. ICI-5, VEH vs. REC-1, ICI-1 vs. ICI-5, and ICI-5 vs. REC-1. In our previous study, ICI-169,369 had a longer time course of effect (38). Therefore, *a priori*, we decided to look at 24-h cumulative REMS and NREMS for ICI-169,369 and 12-h cumulative REMS and NREMS for ICI-170,809. Latencies to NREMS and REMS, and number and duration of REMPs were each analyzed with either a paired *t*-test or a Wilcoxon matched-pair nonparametric analysis (if the data was not normally distributed) with the same *a priori* planned comparisons as for the ANOVAs. NREMS latency was measured with a two-tailed test because no change was expected. REMS measures were measured with one-tailed tests because REMS latency was expected to increase and other REMS measures were expected to decrease.

RESULTS

As shown previously (38), acute oral administration of ICI-170,809 resulted in a significant increase (300% of control, $p < 0.05$, Wilcoxon matched-pair test, one tailed) in the latency to the first REMS episode (Fig. 1a, ICI-1) and a significant suppression of cumulative REMS time for the first 12 h (Fig. 2a), $F(1, 28) = 15.67$, $p < 0.001$. Tests of simple main effects showed significant decreases at 4 ($p < 0.005$) and 6 h ($p < 0.03$). The number of REMPs was significantly decreased ($p < 0.05$, Wilcoxon matched-pair test) and the duration was significantly increased ($p < 0.01$, paired *t*-test) in the first 6 h (Table 1). During the 5 days of 2× daily treatments,

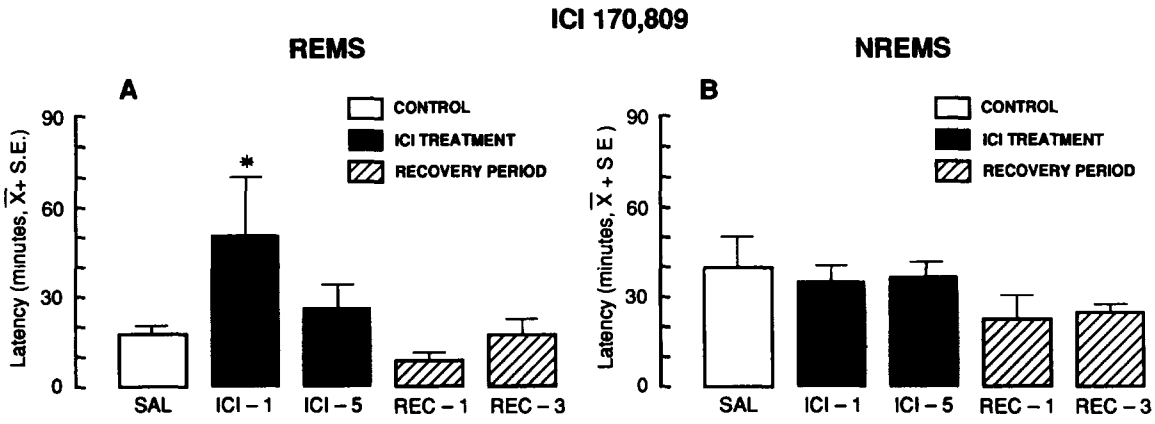

FIG. 1. Latency from initial administration to the onset of REMS (A) and to the onset of NREMS (B) after vehicle control administration, days 1 and day 5 of chronic ICI-170,809 administration (10 mg/kg, p.o., $n = 5$), and days 1 and day 3 of recovery (i.e., sham administrations). *$p < 0.05$, Wilcoxon matched-pair test (one tailed for REMS and two tailed for NREMS).

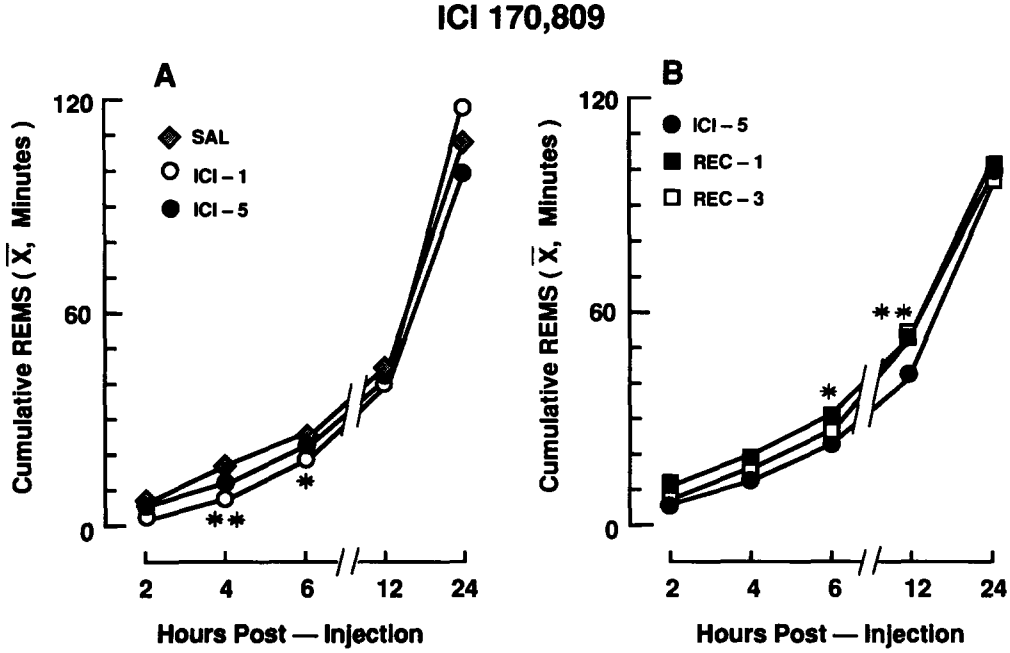

FIG. 2. Effects of chronic p.o. administration of ICI-170,809 on cumulative 24-h REMS. Administrations were at time 0 and at 6 h (not marked on figure). (♦), vehicle control; (○), day 1 of ICI-170,809; (●), day 5 of ICI-170,809; (■), day 1 of recovery; (□), day 3 of recovery. *$p < 0.05$, **$p < 0.01$, simple main effects after ANOVA.

tolerance developed to these effects of ICI-170,809 on REMS. The increase in REMS latency declined from 300 to 150% of control (Fig. 1a, ICI-5). Cumulative REMS time and number and duration of REMPs were not significantly different from predrug levels (Fig. 2a and Table 1). During the first day of the recovery period (i.e., REC-1), a rebound effect was observed, characterized by significant increases in number of REMPs in the first 6 h (Table 1, $p < 0.05$, Wilcoxon matched-pair test, compared to control) and in cumulative REMS for the first 12 h [Fig. 2b, $F(1, 28) = 15.94$, $p < 0.001$, compared to ICI-5] and a nonsignificant decrease in REMS latency (59% of control, Fig. 1a).

Chronic ICI-170,809 had no significant effect on NREMS behavior (Fig. 3a). Although the latency to NREMS decreased and cumulative NREMS increased during the recovery period compared to the last day of ICI-170,809 treatment, these effects were not significant (Figs. 1b and 3b).

Chronic ICI-169,369 had similar effects to those seen with chronic ICI-170,809. On the first day of ICI-169,369 administration, REMS latency was significantly increased (420% of control, $p < 0.01$, paired t-test, one tailed, Fig. 4a). Cumulative REMS for 24 h was significantly decreased (Fig. 5a), $F(1, 63) = 31.73$, $p < 0.0001$. Tests of simple main effects showed significant decreases at h 6 ($p < 0.02$), 12 ($p < 0.001$), and 24 ($p < 0.001$). The number of REMPs was significantly lower at 6 h ($p < 0.001$, paired t-test) but not at 24 h (Table 2). Unlike ICI-170,809, average REMP duration was not significantly increased.

TABLE 1

EFFECT OF ICI 170,809 ON 6-h AND 24-h REM MEASURES

	Total REM Time (min)		Number of REMPs		REMP Duration (min)	
Treatment and Day	6 h	24 h	6 h	24 h	6 h	24 h
Saline	26 ± 5	107 ± 6	17 ± 4	61 ± 6	1.6 ± 0.2	1.8 ± 0.1
ICI-1	19 ± 3	117 ± 5	9 ± 2*	61 ± 2	2.2 ± 0.2†	1.9 ± 0.1
ICI-5	23 ± 5	100 ± 6	14 ± 3	62 ± 3	1.8 ± 0.1	1.6 ± 0.1
Rec-1	33 ± 7	102 ± 6	24 ± 6*	70 ± 6	1.4 ± 0.1	1.5 ± 0.1
Rec-3	28 ± 6	98 ± 5	19 ± 4	61 ± 6	1.5 ± 0.1	1.6 ± 0.1

Data are reported as the means ± SEM. REMP duration refers to the average length of an REM period during the test period.

*$p < 0.05$, †$p < 0.01$ (Wilcoxon matched-pair test for number of REMPs, paired t-test for other comparisons; compared to distilled water control).

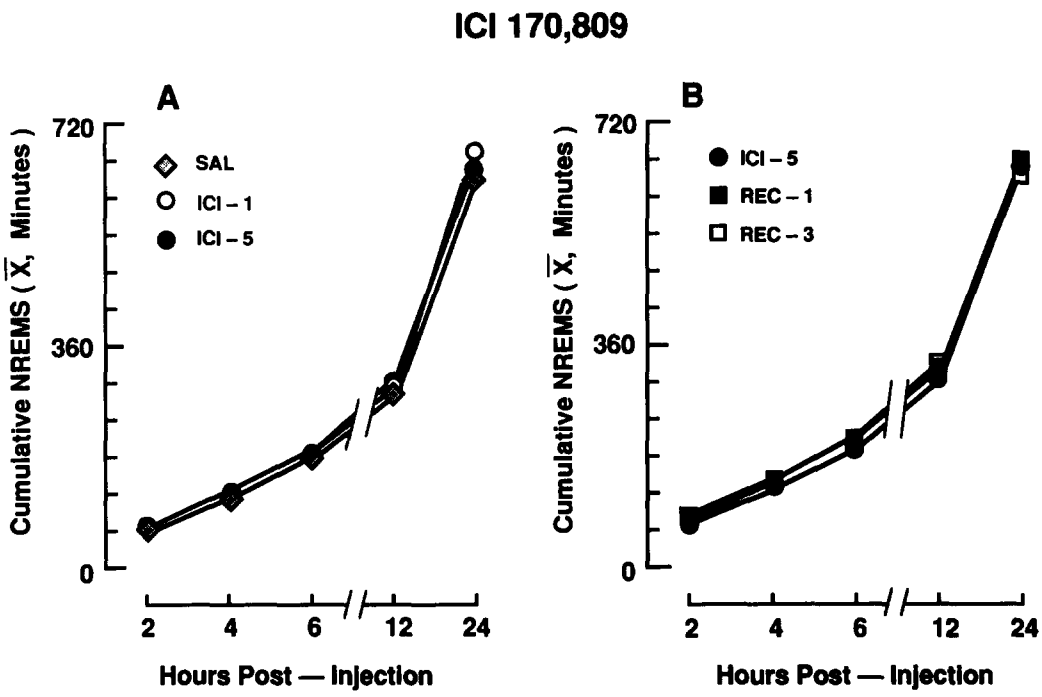

FIG. 3. Effects of chronic p.o. administration of ICI-170,809 on cumulative 24-h NREMS. Administrations were at time 0 and at 6 h (not marked on figure). Same symbols as in Fig. 2.

By day 5, REMS latency was still greater than on the vehicle control day (204% of control), but the difference was not significant ($p > 0.05$, Fig. 4a). However, day 5 REMS latency was significantly lower than day 1 of ICI-169,369 administration ($p < 0.05$, paired t-test, one tailed). Similarly, the number of REMPs was lower on day 5 compared to vehicle, but the difference was not significant (Table 2). Cumulative REMS on day 5 was intermediate between vehicle and day 1 of ICI-169,369 administration (Fig. 5a). Day 5 cumulative REMS was significantly lower than vehicle (Fig. 5a), $F(1, 63) = 10.31$, $p < 0.003$, and significantly higher than day 1 of administration, $F(1, 63) = 4.16$, $p < 0.05$. Tests of simple

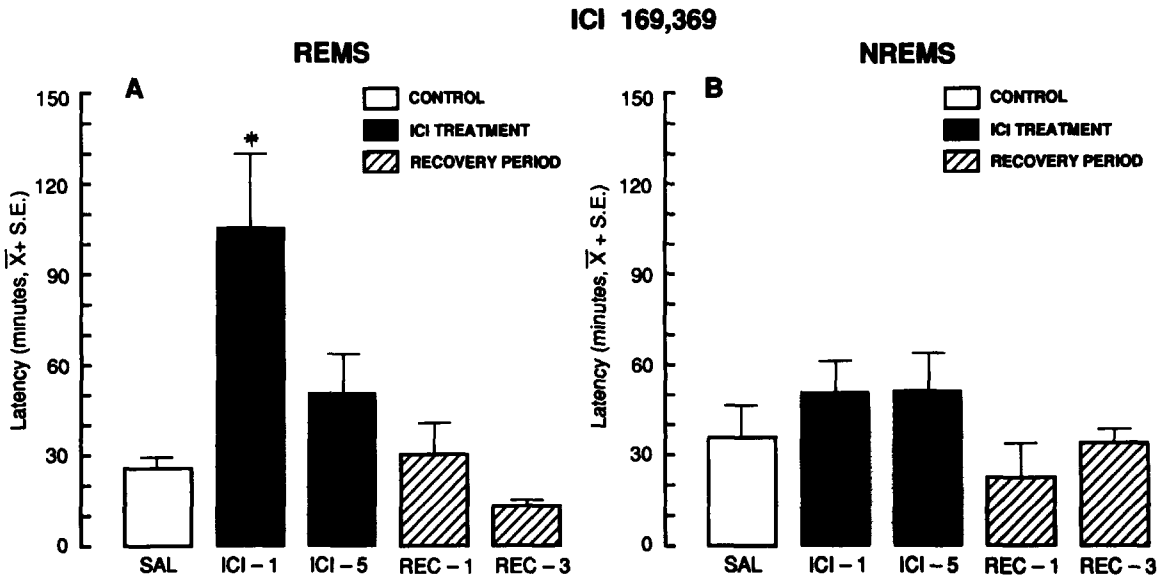

FIG. 4. Latency from initial administration to the onset of REMS (A) and latency to the onset of NREMS (B) after vehicle control, days 1 and 5 of chronic ICI-169,369 administration (40 mg/kg, p.o., $n = 8$), and days 1 and 3 of recovery. *$p <$ 0.05, paired t-test (one tailed for REMS and two tailed for NREMS).

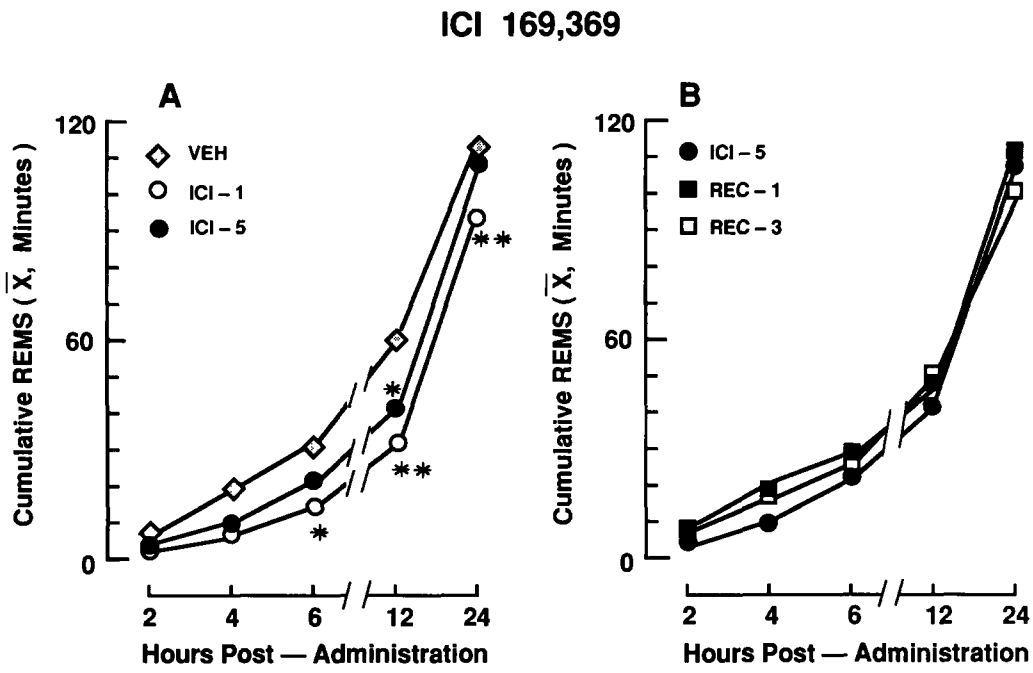

FIG. 5. Effects of chronic p.o. administration of ICI-169,369 on cumulative 24-h REMS. Administrations were at time 0 and at 6 h (not marked on figure). Same symbols as in Fig. 2.

main effects (veh vs. day 5) showed a significant decrease at 12 h after administration ($p < 0.001$).

When ICI-169,369 was discontinued, REMS measures (latency, cumulative amount, and duration) returned to normal (Figs. 4a and 5b and Table 2). No rebound increases were observed.

NREMS latency was nonsignificantly increased on days 1 and 5 of ICI-169,369 administration vs. vehicle (Fig. 4b). Latency was significantly lower on the first day of recovery (no injection) compared to ICI-5 ($p < 0.01$, paired t-test, two tailed). Cumulative NREMS was significantly lower on day 5 of ICI-169,369 administration vs. control, $F(1, 63) = 14.71$, $p < 0.001$, and vs. day 1 of drug administration (Fig. 6a), $F(1, 63) = 4.51$, $p < 0.05$ (fig 6a). The first recovery day was also significantly lower than vehicle, $F(1, 63) = 17.55$, $p < 0.001$, but by the third day of recovery no significant difference was observed (Fig. 6b). The decreases seen in cumulative NREMS were small but consistent.

DISCUSSION

ICI-170,809 strongly suppressed REMS after acute administration. The suppression was characterized by an increase in REMS latency and a decrease in the number of REMPs in the first 6 h. The decrease in REMS was partially compensated for by an increased duration of REMPs. Tolerance to the REMS effects developed so that by the fifth day of administration REMS measures were not significantly different from vehicle control values. When ICI-170,809 was discontinued, a rebound increase in REMS occurred during the first 12 h. A

TABLE 2

EFFECT OF ICI 169,369 ON 6-h AND 24-h REM MEASURES

	Total REM Time (min)		Number of REMPs		REMP Duration (min)	
Treatment and Day	6 h	24 h	6 h	24 h	6 h	24 h
Saline	30 ± 3	111 ± 4	21 ± 2	72 ± 3	1.5 ± 0.1	1.6 ± 0.1
ICI-1	16 ± 3	92 ± 13	8 ± 1†	60 ± 8	1.7 ± 0.1	1.5 ± 0.1
ICI-5	23 ± 3	107 ± 10	15 ± 3	73 ± 5	1.7 ± 0.2	1.5 ± 0.1
Rec-1	29 ± 6	112 ± 13	19 ± 3	69 ± 6	1.5 ± 0.1	1.6 ± 0.1
Rec-3	27 ± 3	100 ± 7	16 ± 2	62 ± 5	1.7 ± 0.1	1.6 ± 0.1

Data are reported as the means ± SEM. REMP duration refers to the average length of an REMP during the test period.

†$p < 0.01$ (paired t-test, compared to distilled water control).

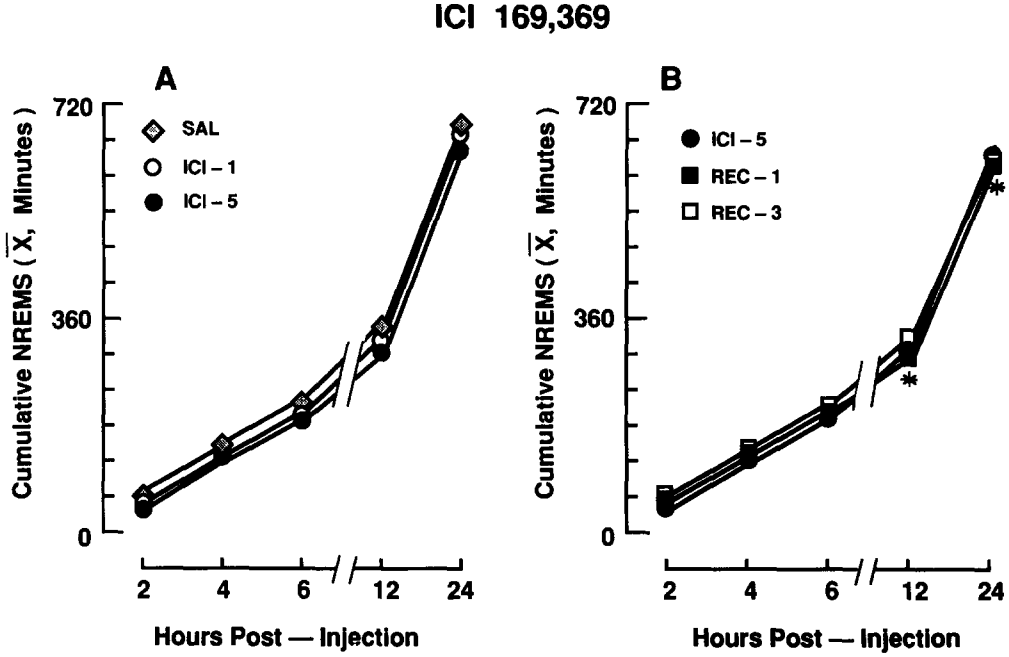

FIG. 6. Effects of chronic p.o. administration of ICI-169,369 on cumulative 24-h NREMS. Administrations were at time 0 and at 6 h (not marked on figure). Same symbols as Fig. 2.

nonsignificant decrease in REMS latency and a significant increase in number of REMPs characterized the rebound. During and after ICI-170,809 administration, no effect was seen on NREMS.

The acute effects of ICI-169,369 were similar to those seen with ICI-170,809, with the exception that average REMP duration was not increased. With chronic administration, tolerance did develop to most of these effects, but cumulative REMS on day 5 of administration remained significantly lower than vehicle control. No rebound increase in REMS was observed when ICI-169,369 was discontinued. Cumulative NREMS was lower than control on day 5 of ICI-169,369 and on the first recovery day, but the differences were small.

In comparing the chronic effects of the two drugs, two differences were observed. First, less tolerance developed to the REMS suppressive effects of ICI-169,369. Second, in contrast to ICI-170,809, no REMS rebound occurred when treatment with ICI-169,369 was discontinued. These effects could be related to the apparent longer time course of ICI-169,369 (38). Alternatively, ICI-170,809 has been reported to be a more potent 5-HT_2 antagonist (11,15) and the differences in receptor potency may account for some of the observed difference in tolerance and the lack of a rebound effect after ICI-169,369. A third possibility that may explain these differences is the recent observation that ICI-169,369 acts as an allosteric activator of the 5-HT_2 receptor system, whereas ICI-170,809 acts as a partial deactivator (11,18).

ICI-169,369 has been reported to be a selective antagonist of 5-HT_2 receptors (2,3,8,18). In in vitro studies, ICI-169,369 has a selectivity ratio of 100 or more for 5-HT_2 vs. 5-HT_{1a}, α_1, α_2, β_1, β_2, dopamine$_2$, muscarinic, and histaminergic binding sites in cortex and isolated tissue studies (3). A similar selectivity has been shown in in vivo studies (2). Dimethylation of ICI-169,369 produces ICI-170,809, which has approximately 20-fold higher affinity for the 5-HT_2 receptor, but retains similar selectivity [Blackburn, unpublished observations; (8,11, 15)]. Recently, both ICI-169,369 and ICI-170,809 have been shown to be antagonists at the 5-HT_{1c} receptor [(32), Blackburn, unpublished observations]. Although tolerance develops to REMS effects of ICI-169,369 and ICI-170,809, no tolerance develops to the ability to block fenfluramine-induced hypothermia after chronic administration for 2 weeks (Blackburn, unpublished observations).

Early research suggested that 5-HT played an important role in the regulation of sleep. Depletion of brain 5-HT by parachlorophenylalanine (PCPA) or electrolytic lesions of the dorsal raphe nucleus, which contains serotonin cell bodies, have been shown to induce a large insomnia (16). However, sleep eventually returned despite continued low levels of brain 5-HT when PCPA was chronically administered (7). In recent years, a number of compounds have been developed that are more specific for 5-HT receptors and, critically, are more selective for the different 5-HT receptor subtypes (26). In the rat, experiments using systemic injections of 5-HT_2 antagonists have consistently shown REMS suppression, whereas effects on NREMS have not been as consistent (5,9,38). Similarly, studies with 5-HT reuptake inhibitors (fluoxetine, indalpine, zimelidine, and alaproclate) have also demonstrated a consistent suppression of REMS and a variable effect on NREMS in the rat (17,25,29,35).

In the present article drugs were administered systemically, so no conclusions can be drawn about site(s) of actions. However, information has begun to accumulate on the cellular pharmacology of serotonin in brain regions thought to play a role in REMS. A recent in vitro study has shown that 5-HT_1 agonists hyperpolarize and 5-HT_2 agonists depolarize separate populations of neurons in the medial pontine reticular forma-

tion, an area thought to play a role in REMS generation (36). Cholinergic neurons in the laterodorsal tegmental nucleus, an area implicated in REMS, have been shown to be hyperpolarized by 5-HT$_1$ agonists (21).

In addition to its role in the regulation of sleep, 5-HT has also been implicated in depressive disorders. Among the reported findings are decreased 5-HT in the postmortem brains of depressed patients and decreased 5-hydroxyindoleacetic acid (5-HIAA) in the cerebrospinal fluid of depressives (22). More recently, the 5-HT$_2$ receptor has been implicated in depression. For example, chronic administration of a wide range of antidepressant drugs has been reported to decrease the number of 5-HT$_2$ receptors in the rat brain (4,13,27,40). Another study showed a significant increase in 5-HT$_2$ binding in the brains of unmedicated depressives compared to matched controls (41).

Not coincidentally, several investigators have proposed that a pathophysiological link may exist between clinical depression and sleep disturbances (30). Depressed patients typically have increased REMS in the first half of the night (characterized by a decreased REM latency and an increased number of rapid eye movements in the first REMP) and a reduction of slow-wave (delta wave) sleep (20,30). Further, most effective antidepressant drugs alter REMS, although many other classes of drugs also alter REMS. Recently, Vogel has done an extensive survey of the literature and reported that many (but not all) antidepressant drugs have three common effects on REMS: a) an initial large suppression of REMS, b) a persistent suppression of REMS, and c) a REMS rebound upon discontinuation of the drug (39). Among the studies supporting their hypothesis are chronic studies in the rat and man showing that both amitriptyline, a classic antidepressant, and zimelidine, a new antidepressant that selectively blocks 5-HT reuptake, share these three characteristic effects on REMS (23,29,34). Although neither ICI-169,369 nor ICI-170,809 share all three of these properties, both drugs acutely produce a large initial REMS suppression, with ICI-170,809 suppressing REMS for 12 h and ICI-169,369 suppressing REMS for 24 h. Tolerance develops to the REMS suppression after chronic administration, with much less tolerance developing to ICI-169,369. An REMS rebound does occur after ICI-170,809 but not after ICI-169,369.

While premature to speculate on the potential clinical usefulness of ICI-170,809 or ICI-169,369 in treating depression, two other 5-HT$_{2/1c}$ antagonists, trazodone and mianserin, have proven effective as antidepressants (6,28). Albeit, the antidepressant mechanism of both drugs is not clear because trazodone's primary metabolite, *m*-chlorophenylpiperazine, is a 5-HT$_{1b/1c}$ agonist and mianserin also acts as an antagonist at α_2 receptors (12). Interestingly, trazodone is one of the four antidepressants that does not have the typical antidepressant effect on REMS (39). In early clinical trials, ritanserin has been reported to be effective for both disthymic disorders and major depressive disorders (19,31), although acute and chronic studies in man have shown no REMS suppression after administration of ritanserin (1,14,24,33). Therefore, the potential usefulness of selective 5-HT$_{2/1c}$ antagonists as antidepressants based upon the REMS mechanism of depression would be highly speculative at this time.

The present results indicate that tolerance develops to the REMS suppressant actions of both ICI-169,369 and ICI-170,809. In a previous study, the acute REMS effects were shown to be dose dependent (38). Together, these data suggest that an influence of serotonin on REMS in rats may involve the 5-HT$_{2/1c}$ receptor subtype. Whether the functional significance of these particular serotonin receptor subtypes resides solely in REMS, rather than in slow-wave sleep or the general control of vigilance, remains to be determined.

REFERENCES

1. Adam, K.; Oswald, I. Effects of repeated ritanserin on middle-aged poor sleepers. Psychopharmacology (Berl.) 99:219–221; 1989.
2. Blackburn, T. P.; Cox, B.; Thornber, C. W.; Pearce, R. J. Pharmacological studies in vivo with ICI-169,369, a chemically novel 5-HT$_2$/5-HT$_{1c}$ receptor antagonist. Eur. J. Pharmacol. 180:229–237; 1990.
3. Blackburn, T. P.; Thornber, C. W.; Pearce, R. J.; Cox, B. In vitro studies with ICI-169,369, a chemically novel 5-HT antagonist. Eur. J. Pharmacol. 150:247–256; 1988.
4. Blackshear, M. A.; Sanders-Bush, E. Serotonin receptor sensitivity after acute and chronic treatment with mianserin. J. Pharmacol. Exp. Ther. 221:303–308; 1982.
5. Borbely, A. A.; Trachsel, L.; Tobler, I. Effect of ritanserin on sleep stages and sleep EEG in the rat. Eur. J. Pharmacol. 156: 275–278; 1988.
6. Brogden, R. N.; Heel, R. C.; Speight, T. M.; Avery, G. S. Trazodone: A review of its pharmacological properties and therapeutic use in depression and anxiety. Drugs 21:401–429; 1981.
7. Dement, W. C.; Mitler, M. M.; Henriksen, S. J. Sleep changes during chronic administration of parachlorophenylalanine. Rev. Can. Biol. 31:239–246; 1972.
8. Doble, A.; Girdlestone, D.; Piot, O.; Allam, D.; Betschart, J.; Boireau, A.; Dupuy, A.; Gueremy, C.; Menager, J.; Zundel, J. L.; Blanchard, J. C. Pharmacological characteristics of RP 62203, a novel 5-hydroxytryptamine 5-HT$_2$ receptor antagonist. Br. J. Pharmacol. 105:27–36; 1992.
9. Dugovic, C.; Wauquier, A.; Leysen, J. E.; Marrannes, R.; Janssen, P. A. J. Functional role of 5-HT-2 receptors in the regulation of sleep and wakefulness in the rat. Psychopharmacology (Berl.) 97:436–442; 1989.
10. Fernstrom, J. D.; Pastel, R. H. The neuropharmacology of serotonin and sleep: An evaluation. In: Koob, G. F.; Ehlers, C. L.; Kupfer, D. J., eds. Animal models of depression. Cambridge, MA: Birkhauser Boston; 1989.
11. Frenken, A. J.; Kaumann, A. J. Dimethylation of the activator ICI-169,369 results in a high-affinity partial deactivator, ICI-170,809, of the arterial 5-hydroxytryptamine$_2$ receptor system. J. Pharmacol. Exp. Ther. 250:707–713; 1989.
12. Fuller, R. W. Pharmacologic properties of serotonergic agents and antidepressant drugs. J. Clin. Psychiatry 48:5–11; 1987.
13. Goodwin, G. M.; Green A. R.; Johnson, P. 5HT$_2$ receptor characteristics in frontal cortex and 5HT$_2$ receptor-mediated head twitch behaviour following antidepressant treatment to mice. Br. J. Pharmacol. 83:235–242; 1984.
14. Idzikowski, C.; Cowen, P. J.; Nutt, D.; Mills, F. J. The effects of chronic ritanserin treatment on sleep and the neuroendocrine response to L-tryptophan. Psychopharmacology (Berl.) 93:416–420; 1987.
15. Jansen, I.; Blackburn, T.; Eriksen, K.; Edvinsson, L. 5-Hydroxytryptamine antagonistic effects of ICI-169,369, ICI-170,809 and methysergide in human temporal and cerebral arteries. Pharmacol. Toxicol. 68:8–13; 1991.
16. Jouvet, M. Indoleamines and sleep-inducing factors. In: Borbely, A. A.; Valatx, J. L., eds. Experimental brain research. Sleep mechanisms. Suppl. 8. New York: Springer-Verlag; 1984:81–94.
17. Kafi-de St. Hilaire, S.; Merica, H.; Gaillard, J.-M. The effects

of indalpine—a selective inhibitor of 5-HT uptake—on rat paradoxical sleep. Eur. J. Pharmacol. 98:413–418; 1984.
18. Kaumann, A. J.; Frenken, M. ICI-169,369 is both a competitive antagonist and an allosteric activator of the arterial 5-hydroxytryptamine$_2$ receptor system. J. Pharmacol. Exp. Ther. 245:1010–1014; 1988.
19. Klieser, E.; Strauss, W. H. Study to establish the indication for the selective S_2 antagonist ritanserin. Pharmacopsychiatry 21: 391–393; 1988.
20. Kupfer, D. J. Interaction of EEG sleep, antidepressants and affective disease. J. Clin. Psychiatry 43:30–35; 1982.
21. Luebke, J. I.; Greene, R. W.; Semba, K.; Kamondi, A.; McCarley, R. W.; Reiner, P. B. Serotonin hyperpolarizes cholinergic low-threshold burst neurons in the rat laterodorsal tegmental nucleus in vitro. Proc. Natl. Acad. Sci. USA 89:743–747; 1992.
22. Meltzer, H. Y.; Lowy, M. T. The serotonin hypothesis of depression. In: Meltzer, H.Y., ed. Psychopharmacology: The third generation of progress. New York: Raven Press; 1987:513–533.
23. Obal, F., Jr.; Benedek, G.; Lelkes, Z.; Obal, F. Effects of acute and chronic treatment with amitriptyline on the sleep–wake activity of rats. Neuropharmacology 24:223–229; 1985.
24. Paiva, T.; Arriaga, F.; Wauquier, A.; Lara, E.; Largo, R.; Leitan, J. N. Effects of ritanserin on sleep disturbances of dysthymic patients. Psychopharmacology (Berl.) 96:395–399; 1988.
25. Pastel, R. H.; Fernstrom, J. D. Short-term effects of fluoxetine and trifluoromethylphenylpiperazine on electroencephalographic sleep in the rat. Brain Res. 436:92–102; 1987.
26. Peroutka, S. J. 5-Hydroxytryptamine receptor subtypes. Annu. Rev. Neurosci. 11:45–60; 1988.
27. Peroutka, S. J.; Snyder, S. H. Regulation of serotonin$_2$ (5-HT$_2$)receptors labeled with [^{3}H]-spiroperidol by chronic treatment with the antidepressant amitriptyline. J. Pharmacol. Exp. Ther. 215:582–587; 1980.
28. Pinder, R. M.; Fink, M. Mianserin. Mod. Probl. Pharmacopsychiatry 18:70–101; 1982.
29. Reyes, R. B.; Hill, S. Y.; Kupfer, D. J. Effects of repeated zimelidine administration on sleep parameters in the rat. Psychopharmacology (Berl.) 88:54–57; 1986.
30. Reynolds, C. F.; Gillin, J. C.; Kupfer, D. J. Sleep and affective disorders. In: Meltzer, H. Y., ed. Psychopharmacology: The third generation of progress. New York: Raven Press; 1987:647–654.
31. Reyntjens, A.; Gelders, Y. G.; Hoppenbrouwers, J. A.; Vanden Bussche, G. Thymosthenic effects of ritanserin (R 55667), a centrally acting serotonin-S_2 receptor blocker. Drug Dev. Res. 8:205–211; 1986.
32. Sahin-Erdemli, I.; Schoeffter, P.; Hoyer, D. Competitive antagonism by recognized 5-HT$_2$ receptor antagonists at 5-HT$_{1c}$ receptors in pig choroid plexus. Naunyn Schmiedeberg Arch. Pharmacol. 344:137–142; 1991.
33. Sharpley, A. L.; Solomon, R. A.; Fernando, A. I.; da Roza Davis, J. M.; Cowen, P. J. Dose-related effects of selective 5-HT$_2$ receptor antagonists on slow wave sleep in humans. Psychopharmacology (Berl.) 101:568–69; 1990.
34. Shipley, J. E.; Kupfer, D. J.; Dealy, R. S.; Griffin, S. J.; Coble, P. A.; McEachran, A. B.; Grochocinski, V. J. Differential effects of amitriptyline and of zimelidine on the sleep electroencephalogram of depressed patients. Clin. Pharmacol. Ther. 36:251–259; 1984.
35. Sommerfelt, L.; Hauge, E. R.; Ursin, R. Similar effect on REM sleep but differential effect on slow wave sleep of the two 5-HT uptake inhibitors zimeldine and alaproclate in cats and rats. J. Neural Trans. 68:127–144; 1987.
36. Stevens, D. R.; McCarley, R. W.; Greene, R. W. Serotonin$_1$ and serotonin$_2$ receptors hyperpolarize and depolarize separate populations of medial pontine reticular formation neurons in vitro. Neuroscience 47:545–553; 1992.
37. Tortella, F. C.; Cowan, A.; Belenky, G. L.; Holaday, J. W. Opiate-like electroencephalographic and behavioral effects of electroconvulsive shock in rats. Eur. J. Pharmacol. 76:121–128; 1981.
38. Tortella, F. C.; Echeverria, E.; Pastel, R. H.; Cox, B.; Blackburn, T. P. Suppressant effects of selective 5-HT-2 antagonists on rapid eye movement sleep in rats. Brain Res. 485:294–300; 1989.
39. Vogel, G. W.; Buffenstein, A.; Minter, K.; Hennessey, A. Drug effects on REM sleep and on endogenous depression. Neurosci. Biobehav. Rev. 14:49–63; 1990.
40. Wamsley, J. K.; Byerley, W. F.; McCabe, R. T.; McConnell, E. J.; Dawson, T. M.; Grosser, B. I. Receptor alterations associated with serotonergic agents: An autoradiographic analysis. J. Clin. Psychiatry 48:19–25; 1987.
41. Yates, M.; Leake, A.; Candy, J. M.; Fairbairn, A. F.; McKeith, I. G.; Ferrier, I. N. 5HT$_2$ receptor changes in major depression. Biol. Psychiatry 27:489–496; 1990.