

# Effect of SX-3228, a selective ligand for the BZ<sub>1</sub> receptor, on sleep and waking during the light-dark cycle in the rat

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## Abstract

The effects of the benzodiazepine<sub>1</sub> (BZ<sub>1</sub>) receptor agonist SX-3228 were studied in rats (N = 12) implanted for chronic sleep procedures. Administration of 0.5, 1.0 and 2.5 mg/kg SX-3228, *sc*, to rats 1 h after the beginning of the light phase of the light-dark cycle induced a significant reduction of rapid-eye-movement sleep (REMS) during the third recording hour. Moreover, slow wave sleep (SWS) was increased during the fourth recording hour after the two largest doses of the compound. Administration of 0.5, 1.0 and 2.5 mg/kg SX-3228 one hour after the beginning of the dark period of the light-dark cycle caused a significant and maintained (6-h recording period) reduction of waking (W), whereas SWS and light sleep (LS) were increased. REMS values tended to increase during the entire recording period; however, the increase was statistically significant only for the 1.0 mg/kg dose during the first recording hour. In addition, a significant and dose-related increase of power density in the delta and the theta regions was found during nonREM sleep (LS and SWS) in the dark period. Our results indicate that SX-3228 is a potent hypnotic when given to the rat during the dark period of the light-dark cycle. Moreover, the sleep induced by SX-3228 during the dark phase closely resembles the physiological sleep of the rat.

## Key words

- SX-3228
- Benzodiazepine<sub>1</sub> receptor
- Sleep
- Waking
- REM sleep
- Zolpidem
- Light-dark cycle

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## Introduction

Gamma-aminobutyric acid (GABA) is the most important inhibitory transmitter in the central nervous system (CNS). GABA is released from GABAergic neurons and binds to both GABA<sub>A</sub> and GABA<sub>B</sub> receptors (1). The GABA<sub>A</sub> receptor is a protein that contains specific binding sites for GABA, benzodiazepines (BZD), barbiturates, neurosteroids and convulsants. The interaction of com-

pounds from each of these classes with their respective but distinct modulatory sites on GABA<sub>A</sub> receptors allosterically regulates GABA-induced chloride currents (2). Two subclasses of BZD receptors, termed BZ<sub>1</sub> and BZ<sub>2</sub>, are of relevance in regard to a series of compounds with hypnotic properties (3). Benzodiazepine hypnotics and zopiclone, a non-BZD hypnotic, do not discriminate between these BZ-receptor subtypes. In contrast, zolpidem displays much higher affinity

for the BZ<sub>1</sub> than the BZ<sub>2</sub> site. The relative potency of zolpidem, an imidazopyridine hypnotic, to displace [<sup>3</sup>H]-flunitrazepam from the cerebellum (BZ<sub>1</sub>-site) was 5.5 nM (IC<sub>50</sub>), while that corresponding to the dentate gyrus (BZ<sub>2</sub>-site) was 400 nM (4).

A compound with similar selectivity, SX-3228 [6-benzyl-3-(5-methoxy-1,3,4-oxadiazol-2-yl)-5,6,7,8-tetrahydro-1,6-naphthyridin-2 (1H) one], has been shown to bind to BZ receptors, but not to dopamine (D<sub>1</sub>, D<sub>2</sub>), serotonin (5-HT<sub>1</sub>, 5-HT<sub>2</sub> and 5-HT<sub>3</sub> subtypes), noradrenaline (α<sub>1</sub>, α<sub>2</sub>, β), GABA or acetylcholine (muscarinic) subtypes. Among the BZ-receptor subtypes, SX-3228 preferentially binds to the BZ<sub>1</sub> receptor (cerebellum: IC<sub>50</sub> = 17 nM). It has very weak affinity for the BZ<sub>2</sub> receptor (spinal cord: IC<sub>50</sub> = 127 nM), and virtually no affinity for the peripheral type BZ receptor (kidney: IC<sub>50</sub> >10.000 nM) (5). Pretreatment with SX-3228 antagonized pentylenetetrazole-induced myoclonic convulsions in mice and rats, decreased locomotor activity in mice, and mitigated attack responses in cynomolgus monkeys. Tolerance did not develop to the antipentylenetetrazole effect of SX-3228 (6).

Earlier studies with the BZDs midazolam and triazolam, and with zopiclone and zolpidem have demonstrated differences in their pharmacological profiles. Thus, whereas the BZDs and zopiclone are effective in blocking pentylenetetrazole-induced convulsions at very low doses, the sedative and sleep-inducing effect of zolpidem is exerted at doses that are 10-20 times lower than those needed for anticonvulsant or myorelaxant activities (7). Available evidence suggests that SX-3228 possesses a similar profile (8).

Attempts have been made to test the effects of drugs with putative hypnotic activity in situations in which sleep is impaired by nonpharmacological or pharmacological procedures. Nonpharmacological models have included the induction of insomnia by environmental changes (the animal is moved to a

novel individual cage or is connected for the first time to the recording cables), or by decreasing the duration of the dark period (16-h light:8-h dark) in animals kept previously on a 12-h light:12-h dark cycle (9,10). Pharmacological procedures have included the injection of amphetamine or caffeine (11,12). All these procedures generate considerable amounts of stress and alter a critical balance between neurotransmitter systems at central sites.

In order to circumvent these limitations, we tested the effects of compound SX-3228 in animals either sleeping *ad libitum* (light period) or being predominantly awake (dark period). Since the increase in sleep time induced by CNS depressants is inversely related to the baseline values, characterization of SX-3228 during the dark period constitutes an adequate approach to test its hypnotic action.

The present study was designed to quantify the effects of SX-3228 on sleep and waking (W) in the rat. For this purpose, compound SX-3228 was given over a range of doses at the beginning of either the light or the dark phase to rats prepared for chronic sleep recordings.

## Material and Methods

Twelve male Wistar rats (School of Medicine Breeding Laboratories, Montevideo, Uruguay) weighing 350-380 g were implanted with Nichrome electrodes (200 μm in diameter) under general anesthesia with 40.0 mg/kg sodium pentobarbital for chronic sleep recordings from the frontal and occipital cortices and from the dorsal neck musculature. The animals were housed individually in a temperature-controlled room (22 ± 1°C), with food and water provided *ad libitum*. One group of animals was kept on a 12-h light:12-h dark cycle (lights on at 7.00 a.m. and off at 7.00 p.m.), while the second group was housed on a 12-h dark:12-h light cycle (lights off at 7.00 a.m. and on at 7.00 p.m.).

The period of adjustment of the second group of rats used in our study lasted 4 weeks and was followed by a stable cycle of W and sleep (13). Both groups of animals were habituated for 4 days to a chamber fitted with slip-rings and cable connectors and to the injection procedure. Thereafter, they were given either a control solution or the drug to be tested.

### Experiment 1

We studied the effects of SX-3228 (Dainippon Pharmaceutical, Osaka, Japan) 0.5-2.5 mg/kg in one group of animals (N = 6) during the light phase of the 12-h light:12-h dark cycle, starting 1 h after the beginning of the light period. Polysomnographic recordings were started immediately after control solution or drug administration. Each rat received all four treatments (control, and 0.5, 1.0, 2.5 mg/kg SX-3228).

### Experiment 2

We examined the effects of 0.5-2.5 mg/kg SX-3228 in animals (N = 6) adapted to a 12-h dark:12-h light cycle for 4 weeks, starting 1 h after the beginning of the dark period. Each animal received all four treatments (control, and 0.5, 1.0, 2.5 mg/kg SX-3228).

### Procedures

SX-3228 was mixed with a small volume of Tween-80 and suspended in saline. Subcutaneous (*sc*) injections were given in a final volume of 1.0 ml/kg. All rats were given the corresponding volume of control solution (saline + Tween-80) in the control sessions. Following *sc* injection, a 6-h sleep recording was started at approximately 8:00 a.m. At least 4 days were allowed to elapse between injections to avoid long-lasting and rebound effects on sleep.

### Data analysis

The electrographic activity of 25-s epochs was analyzed in a blind manner and assigned to the following categories based on the waveform: W, light sleep (LS), slow wave sleep (SWS), and rapid-eye-movement sleep (REMS). SWS and REMS latencies were also determined (14). In the group of animals maintained on a 12-h dark:12-h light cycle, one EEG signal derived from the frontal cortex was filtered, digitized at a sampling of 128 Hz, and saved to an optical disk. A time code was also written every 20 s on both the paper tracing and the disk. Time code-delimited epochs enabled precise correspondence between the visual scoring of sleep recordings and the computer analysis. Epochs containing artifacts in the EEG lead were eliminated from the analysis. Data saved to the optical disk corresponding to non-REMS (LS and SWS) episodes were analyzed off-line in the range of 0.3-30 Hz with PASS PLUS (Delta Software, St. Louis, MO, USA) spectral analysis software.

### Statistical analysis

One-way analysis of variance with multiple measures was used for statistical comparison of three or more samples, with multiple *post-hoc* comparisons performed with the Newman-Keuls test when ANOVA was significant ( $P < 0.05$ ).

## Results

### Experiments carried out during the light phase

Figure 1 and Table 1 show that administration of 0.5-2.5 mg/kg SX-3228 to rats during the light phase induced a significant reduction of REMS ( $P < 0.05$ ) during the third recording hour. In addition, REMS latency was significantly increased after the highest dose of the BZ<sub>1</sub> agonist and SWS was in-

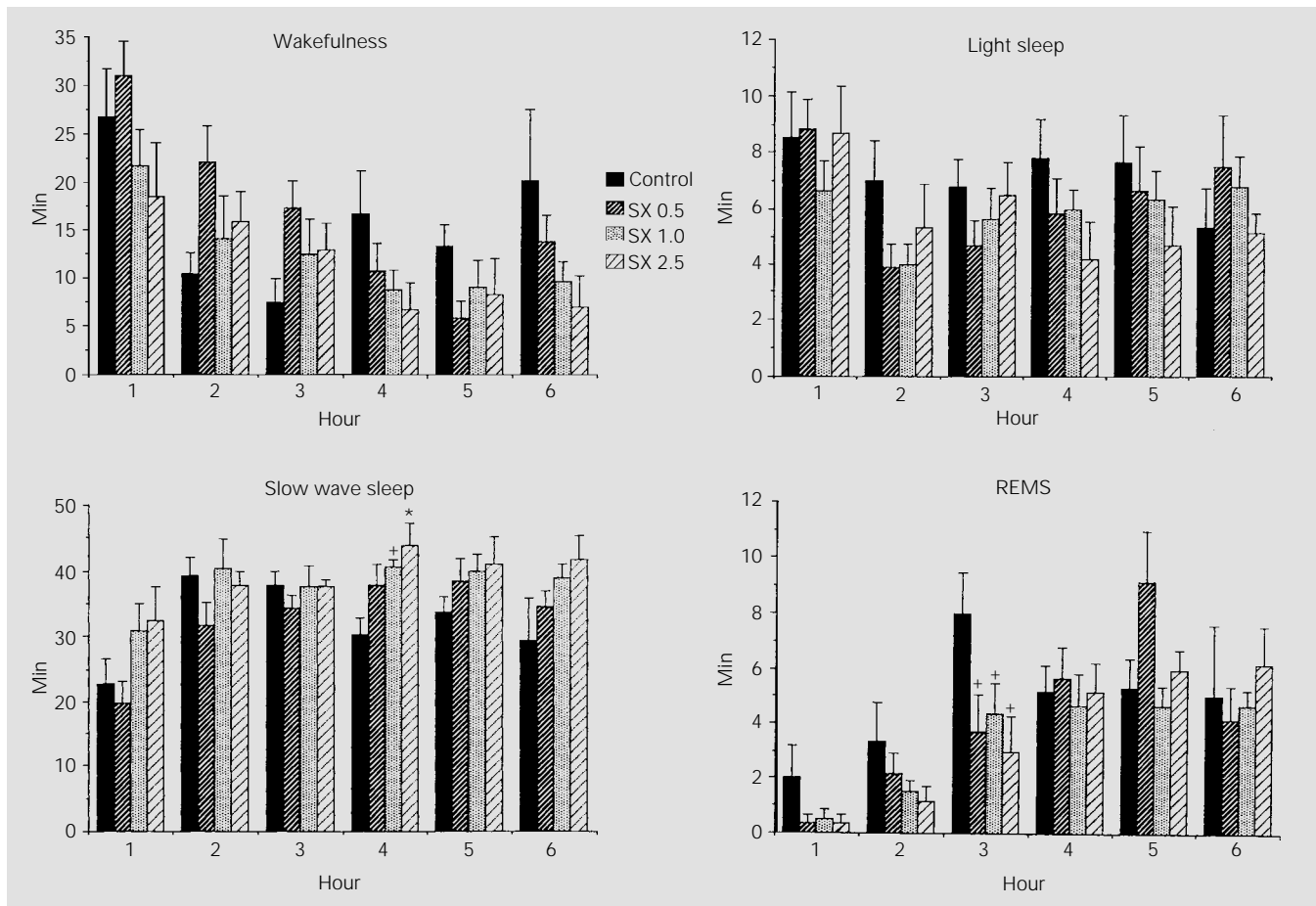


Figure 1 - The effect of SX-3228 (SX) on wakefulness, light sleep, slow wave sleep and rapid-eye-movement sleep (REMS) during the light phase of the light-dark cycle. Ordinate: mean amount in minutes of behavioral state according to EEG criteria. All values are the means (min)  $\pm$  SEM collected at 1-h intervals. Six animals were in each experimental group. Doses are reported in mg/kg. \* $P < 0.05$ ; \* $P < 0.01$  compared to control (Newman-Keuls test).

Table 1 - Effect of SX-3228 on sleep latencies during the light phase of the light-dark cycle.

Data are reported as means  $\pm$  SEM. Six animals were in each experimental group. The doses are reported as mg/kg. \* $P < 0.05$  compared to control (Newman-Keuls test). REMS, Rapid-eye-movement sleep.

	Slow wave sleep latency (min)	REMS latency (min)
Control	9.8 $\pm$ 2.2	65.3 $\pm$ 17.7
SX-3228		
0.5	9.2 $\pm$ 2.0	61.8 $\pm$ 9.1
1.0	4.8 $\pm$ 2.2	85.5 $\pm$ 17.9
2.5	7.7 $\pm$ 3.4	104.5 $\pm$ 13.5*

increased ( $P < 0.05-0.01$ ) during the fourth recording hour after the two largest doses of the compound.

#### Experiments carried out during the dark phase

Control sessions performed during the dark phase showed that the animals remained awake significantly longer and slept less than in control sessions performed during the light phase with the first group of animals. Administration of SX-3228 (0.5-2.5 mg/kg) at the beginning of the dark period significantly and dose-dependently reduced W and increased SWS during the 6-h recording pe-

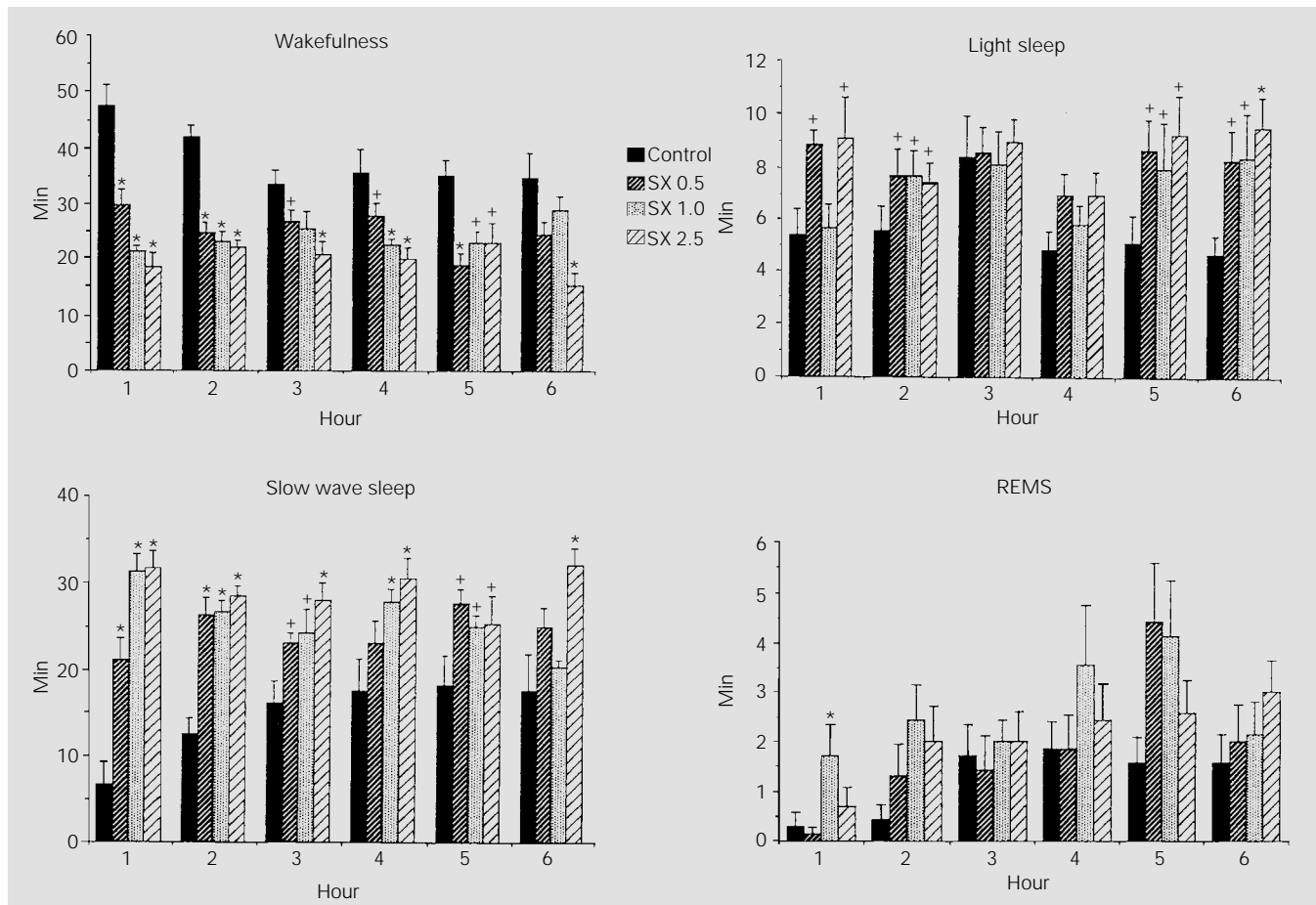


Figure 2 - The effect of SX-3228 (SX) on sleep and waking during the dark phase of the light-dark cycle. All values are the means (min)  $\pm$  SEM collected at 1-h intervals. Six animals were in each experimental group. Doses are reported in mg/kg. \* $P$ <0.05; \*\* $P$ <0.01 compared to control (Newman-Keuls test). REMS, Rapid-eye-movement sleep.

riod ( $P$ <0.05-0.01) (Figure 2); however, significant changes during the last recording hour were restricted to the 2.5 mg/kg dose ( $P$ <0.01). The quantity of LS also increased significantly during the first, second, fifth and sixth recording hours. REMS values tended to augment during the entire recording period; however, the increase attained significance ( $P$ <0.01) only with the 1.0 mg/kg dose during the first recording hour. As shown in Table 2, there was an overall decrease of SWS and REMS latencies during the dark phase, which attained significance ( $P$ <0.05-0.01) with the two largest doses.

The effect of SX-3228 on EEG power density in nonREMS during the dark phase

Table 2 - Effect of SX-3228 on sleep latencies during the dark phase of the light-dark cycle.

Values are reported as means  $\pm$  SEM. Six animals were in each experimental group. The doses are reported as mg/kg. \* $P$ <0.05; \*\* $P$ <0.01 compared to control (Newman-Keuls test). REMS, Rapid-eye-movement sleep.

	Slow wave sleep latency (min)	REMS latency (min)
Control	22.6 $\pm$ 6.2	141.3 $\pm$ 38.5
SX-3228		
0.5	8.7 $\pm$ 2.7	119.4 $\pm$ 21.4
1.0	4.9 $\pm$ 2.2*	52.7 $\pm$ 17.0**
2.5	5.9 $\pm$ 2.2*	71.7 $\pm$ 22.6*

is shown in Figure 3. The values are expressed for each frequency band relative to the corresponding value of the control night (= 100%). The spectrum computed for nonREMS (light sleep and slow wave sleep) of the 6-h recording period revealed a significant increase ( $P < 0.05$ ) of power density in the low frequency range (delta = 0.3-3 Hz; theta = 4-6 Hz) after the 2.5 mg/kg dose. Significant changes after the 0.5 and 1.0 mg/kg dose were restricted to the 1-3 Hz and 0.3-3 Hz band, respectively. Moreover, values corresponding to higher frequencies (alpha = 8-12 Hz; sigma = 12-15 Hz; beta = 23-30 Hz) did not differ significantly from the control levels.

## Discussion

Injection of compound SX-3228 at the beginning of the light period increased SWS during the fourth hour after treatment, and suppressed REMS during the third hour of recording. Administration of SX-3228 at the beginning of the dark period gave rise to a significant and maintained (6-h recording

period) reduction of W, whereas SWS and LS were augmented. Moreover, SWS and REMS latencies became significantly smaller after injection of the 1.0 or the 2.5 mg/kg dose of the compound. In addition, a significant and dose-dependent increase of power density in the delta and theta regions was found during the administration of SX-3228. On the other hand, the relative EEG power density corresponding to the alpha, sigma and beta bands showed no significant changes.

Thus, the effect of SX-3228 on sleep in animals sleeping *ad libitum* was modest and short-lived. In contrast, SX-3228 induced a robust and maintained increase of nonREMS in predominantly awake animals. In other words, under our experimental conditions the sleep-inducing and maintaining effects of SX-3228 were mainly evident when the compound was given at the beginning of the dark period.

In the study by Kurumiya et al. (8) in which SX-3228 (0.3-3.0 mg/kg, *po*) was given to rats during the light period, SWS was increased and LS was reduced during the 12-h recording period. REMS showed an initial decrement (first 4 h) after the highest dose. In addition, SX-3228 (1.0 or 5.0 mg/kg, *po*) produced an increase in power in the low frequency band (1-4 Hz). The relatively large amounts of SWS observed by Kurumiya et al. (8) after injection of SX-3228 during the light phase could be due to the limited duration of the habituation period (1 h) of the experimental animals to the recording chamber. Thus, the authors may have induced a nonpharmacological insomnia in their rats.

In cortical recordings of curarized rats, zolpidem induced sleep periods of 10 to 60 min duration with doses in the range of 0.1 to 1.0 mg/kg, *ip*. Power spectral analysis of the electrocorticogram during zolpidem administration showed a dominant peak in the delta frequency band (1-4 Hz) (15).

Administration of the BZD hypnotics

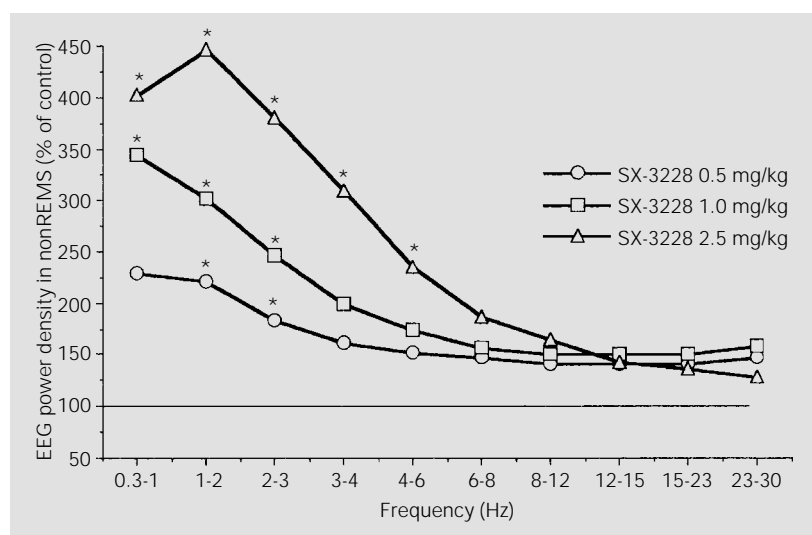


Figure 3 - Effect of SX-3228 on the mean EEG power density of nonREMS (non-rapid-eye-movement sleep) (relative to baseline) during the dark period of the light-dark cycle. The curves indicate mean values of successive frequencies which are reported as percent of the placebo reference value (= 100%) during a 6-h period. SX-3228 significantly and dose-dependently increased power density in the delta and theta regions ( $^*P < 0.05$ ; Newman-Keuls test).

midazolam (0.1-1.0 mg/kg, *ip*) or triazolam (0.03-0.3 mg/kg, *ip*) to curarized rats induced the appearance of frequent bursts of sleep spindles, particularly in the sensorimotor cortex. Sequential spectral analysis showed that both compounds preferentially affected frequencies of 12 to 14 Hz (8,16).

When given to freely moving rats during the light phase, zolpidem (1.0-3.0 mg/kg, *po*) failed to alter sleep patterns. At much higher doses (10.0 mg/kg, *po*), zolpidem significantly increased SWS and delayed the appearance of REMS (16). As compared to zolpidem, midazolam (10.0 mg/kg, *po*) also induced an increase of SWS without disrupting REMS. On the other hand, nitrazepam (3.0 mg/kg, *po*) induced no significant changes in sleep or waking (16,17).

Thus, administration of zolpidem to curarized animals, or of compound SX-3228 to freely moving rats during the dark phase increased SWS and energy peaks in the 1- to 4-Hz frequency band, which is typical of physiological sleep (8,18). On the other hand, midazolam or triazolam induced the appearance of sleep spindles in the cortical EEG, and a large increase in energy between 12 and 14 Hz when given to freely moving rats. Thus, unlike the sleep induced by BZD, the sleep induced by either zolpidem or SX-3228 closely resembles physiological sleep in the rat.

What are the mechanisms involved in the SX-3228-induced increase of nonREMS and REMS during the dark period of the light-dark cycle? As mentioned before, SX-3228 preferentially binds to BZ<sub>1</sub> receptor and increases GABA-induced chloride currents (2). Gamma-aminobutyric acid is the most important inhibitory transmitter in the CNS, and has been proposed to play an essential role in the generation of nonREMS (19). In this respect, the GABA<sub>A</sub> agonist gaboxadol promoted nonREMS and enhanced delta activity in the rat (20). In addition, the highly selective GABA<sub>A</sub> agonist muscimol dose-

dependently increased both nonREMS and REMS and enhanced delta and sigma activity (21).

Pertinent to our discussion is the reciprocal interaction hypothesis of REMS generation proposed by McCarley and Hobson (22), which identifies cholinergic neurons in the laterodorsal and pedunculopontine tegmental nuclei as promoting REMS, and their inhibition by serotonergic (5-HT) afferents from the dorsal raphe nucleus (DRN) and noradrenergic afferents from the locus coeruleus (LC). The activity of DRN serotonergic neurons and LC noradrenergic neurons is at its highest during W, it diminishes during SWS and is virtually suppressed when the animal starts REMS (23). It has been proposed that a GABA input from the reticular formation could inhibit DRN and LC cells during REMS. In this respect, Nitz and Siegel (24,25) described higher GABA levels during REMS compared with W at the DRN and LC site. Reports indicating that direct microinjection of the GABA<sub>A</sub> receptor agonist muscimol into the DRN increases REMS, whereas microinjection of the GABA<sub>A</sub> receptor antagonists bicuculline and picrotoxin induce opposite effects, further support the participation of GABA in the inhibition of 5-HT neurons and facilitation of REMS production (24).

In the past, animal data obtained after administration of BZD hypnotics predicted clinical effects in humans with primary insomnia and insomnia related to anxiety disorders. Thus, it could be worthwhile to test the effect of SX-3228 in patients with difficulty in initiating or maintaining sleep.

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## References

- Macdonald RL & Olsen RW (1994). GABA<sub>A</sub> receptor channels. *Annual Review of Neuroscience*, 17: 569-602.
- Besnard F, Avenet P, Itier V, Granger P, Partisetti M, Depoortere H, Graham D & Langer SZ (1996). GABA<sub>A</sub> receptor subtypes and the mechanism of action of zolpidem. In: Freeman H, Puech AJ & Roth T (Editors), *Zolpidem: An Update of its Pharmacological Properties and Therapeutic Place in the Management of Insomnia*. Elsevier, Paris, 21-31.
- Muller WE (1995). Pharmacology of the GABAergic/benzodiazepine system. In: Kales A (Editor), *Pharmacology of Sleep*. Springer, Berlin, 211-242.
- Langer SZ, Arbilla S, Benavides J, Depoortere H & Scatton B (1990). Receptors involved in the mechanism of action of the imidazopyridine zolpidem. In: Barthouil P (Editor), *Insomnie et Imidazopyridines*. Excerpta Medica, Amsterdam, 207-218.
- Furukawa K, Oka M, Kohayakawa H, Kurumiya S, Saruwatari T, Ono K, Odai O & Karasawa T (1994). SX-3228, a putative nonbenzodiazepine hypnotic. I. Neuropharmacological profile. *Neuropsychopharmacology*, 10 (Suppl): 227S (Abstract P:174-60).
- Oka M, Furukawa K, Noda Y, Ochi Y, Kitazono Y, Ono K, Odai O & Karasawa T (1994). SX-3228, a putative non-benzodiazepine hypnotic. II. Behavioral effects. *Neuropsychopharmacology*, 10 (Suppl): 227S (Abstract P:174-61).
- Zivkovic B, Perrault G, Morel E & Sanger DJ (1990). Neuropsychopharmacological profile of zolpidem, a new imidazopyridine hypnotic. In: Barthouil P (Editor), *Insomnie et Imidazopyridines*. Excerpta Medica, Amsterdam, 219-230.
- Kurumiya S, Nagai R, Furukawa K, Oka M & Karasawa T (1994). SX-3228, a putative non-benzodiazepine hypnotic. II. Electroencephalographic and sleep studies. *Neuropsychopharmacology*, 10 (Suppl): 228S (Abstract P:174-62).
- Halperin JM, Miller D & Iorio LC (1981). Sleep inducing effects of three hypnotics in a new model of insomnia in rats. *Pharmacology, Biochemistry and Behavior*, 14: 811-814.
- Michaud JC, Muiyard JP, Capdevielle G, Ferran E, Geiordano-Orsini JP, Veyrun J, Roncucci R & Mouret J (1982). Mild insomnia induced by environmental perturbations in the rat: A study of this new model and of its possible applications in pharmacological research. *Archives Internationales de Pharmacodynamie et de Therapie*, 259: 93-105.
- Monti JM (1979). The effects of neuroleptics with central dopamine and noradrenaline receptor blocking properties in the l-dopa and (+)-amphetamine-induced waking EEG in the rat. *British Journal of Pharmacology*, 67: 87-91.
- Radulovacki M, Miletich RS & Green RD (1982). N<sup>6</sup> (L-phenylisopropyl)adenosine (L-PIA) increases slow-wave sleep (S<sub>2</sub>) and decreases wakefulness in rats. *Brain Research*, 246: 178-180.
- Grant L, Hopkinson P, Jennings G & Jenner FA (1971). Period of adjustment of rats used for experimental studies. *Nature*, 232: 135.
- Monti JM, Hawkins M, Jantos H, Labraga P & Fernandez J (1988). Biphasic effects of dopamine D-2 receptor agonists on sleep and wakefulness in the rat. *Psychopharmacology*, 95: 395-400.
- Arbilla S, Depoortere H, George P & Langer SZ (1985). Pharmacological profile of the imidazopyridine zolpidem at benzodiazepine receptors and electrocortico-gram in rats. *Archives of Pharmacology*, 330: 248-251.
- Depoortere H, Zivkovic B, Lloyd KG, Sanger DJ, Perrault G, Langer SZ & Bartholini G (1986). Zolpidem, a novel nonbenzodiazepine hypnotic. I. Neuropharmacological and behavioral effects. *Journal of Pharmacology and Experimental Therapeutics*, 237: 649-658.
- Depoortere H & Granger P (1985). Differentiation of EEG sleep stages in the rat and the cat by automatic analysis. In: Kubicki S & Herrmann WM (Editors), *Methods of Sleep Research*. Gustav Fischer, Stuttgart, 37-45.
- Depoortere H (1985). A comparative EEG study of some benzodiazepine (BZD) and non-BZD sedative or hypnotic drugs. *Pharmacopsychiatry*, 18: 17-19.
- McCormick DA (1992). Neurotransmitter actions in the thalamus and cerebral cortex and their role in neuromodulation of thalamocortical activity. *Progress in Neurobiology*, 39: 337-388.
- Lancel M & Faulhaber J (1996). The GABA<sub>A</sub> agonist THIP (gaboxadol) increases non-REM sleep and enhances delta activity in the rat. *Neuroreport*, 7: 2241-2245.
- Lancel M, Crönlein TAM & Faulhaber J (1996). Role of GABA<sub>A</sub> receptors in sleep regulation: Differential effects of muscimol and midazolam on sleep in rats. *Neuropsychopharmacology*, 15: 63-74.
- McCarley RW & Hobson JA (1975). Neuronal excitability modulation over the sleep cycle: a structural and mathematical model. *Science*, 189: 58-60.
- Trulson ME & Jacobs BL (1979). Raphe unit activity in freely moving cats: correlation with level of behavioral arousal. *Brain Research*, 163: 135-150.
- Nitz D & Siegel J (1997). GABA release in the dorsal raphe nucleus: role in the control of REM sleep. *American Journal of Physiology*, 273: R451-R455.
- Nitz D & Siegel J (1977). GABA release in the locus coeruleus as a function of sleep/wake state. *Neuroscience*, 78: 795-801.