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# Effects of Nisoxetine, a Selective Noradrenaline Transporter Blocker, on Sleep in Rats

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PYTHON, A., Y. CHARNAY, R. MIKOLAJEWSKI, H. MERICA AND Z. DE SAINT HILAIRE. *Effects of nisoxetine, a selective noradrenaline transporter blocker, on sleep in rats.* PHARMACOL BIOCHEM BEHAV **58**(2) 369–372, 1997.—Nisoxetine has been shown to block specifically noradrenaline (NA) reuptake. Therefore, this potential antidepressant is a valuable tool for investigating the involvement of the NA system in sleep regulation. This study aimed to investigate the effects of different doses of nisoxetine on sleep parameters in rats. The main effects were observed with the highest dose and concern paradoxical sleep (PS). Indeed, although total sleep time was not modified, PS appeared later and its amount and the number of its episodes were reduced. These changes suggest a critical involvement of NA in the induction of PS. © 1997 Elsevier Science Inc.

Nisoxetine Sleep Rat

BRAIN STEM noradrenergic systems may constitute an important way of regulating sleep mechanisms (11,25). Electrophysiological studies in animals have shown that the firing of noradrenergic neurons of the locus coeruleus (LC) decreased during slow wave sleep (SWS) and nearly ceased during paradoxical sleep (PS) (10). These observations led to the hypothesis of an inhibitory influence of the LC noradrenergic neurons on PS generation. Moreover, intracerebral administration of noradrenaline (NA) produced arousal in rats, suggesting a tonic activity of the noradrenergic system in the maintenance of waking (W) (5). In fact, the decreased firing of NA neurons (PS-off neurons) seems to play a permissive role in the induction and maintenance of PS (9).

Tricyclic antidepressants are often mixed monoamine uptake blockers (12). They inhibit uptake of monoamines and then their inactivation. They decrease monoamine turnover and diminish PS. Nisoxetine, (±)-γ-(2-methoxyphenoxy)-*N*-methyl-benzeneprop-anamine, a selective marker of the NA transporter, inhibited high-affinity uptake of NA into synaptosomes isolated from rat brain (2,3,29). This potential antidepressant (28) protected rat hypothalamic neurons from 6-hydroxydopamine neurotoxicity in vivo, also suggesting a blockage of NA uptake (27). Altogether, these data suggest that nisoxetine is a specific NA reuptake inhibitor. When administered orally to cats, nisoxetine produced a diminution of PS, which consistent with the inhibitory role of NA neurons in PS (23). Consequently, the present study was designed to test whether nisoxetine, a potent inhibitor of NA uptake, can affect sleep, particularly PS, in rats.

## MATERIALS AND METHODS

Eight male Wistar rats weighing 250–300 g were anaesthetised with nembutal (55 mg/kg intraperitoneally) and surgically implanted with two electrodes for cortical electroencephalogram (EEG) recordings (fronto-occipital derivation) and two electrodes for electromyogram (EMG) recordings from the neck muscles. After surgery, the animals were housed in individual cages and allowed 15 days to recover. They had free access to food and water and were kept under constant temperature (25°C) and humidity (55%) conditions on a 12 L:12 D cycle (lights on at 0800 h). Three days before recordings, the rats were placed in recording cages and connected via a cable to a rotating collector.

The recordings lasted from 0800 to 1400 h. The EEG recording appliance was Grass model 78D, with a 50-mV ampli-

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TABLE 1
EFFECTS OF NISOXETINE ON SLEEP VARIABLES DURING THE 6 h OF RECORDING

| Variable | NaCl | Dose of nisoxetine (mg/kg) 0.05 | 0.1 | 0.25 | 1 |
|---|---|---|---|---|---|
| Sleep (min) | 245.7 ± 28.9 | 254 ± 19.9 | 250.4 ± 27.4 | 249.4 ± 14.5 | 252.4 ± 18.8 |
| Waking (min) | 114.3 ± 28.9 | 106 ± 19.9 | 109.6 ± 27.4 | 110.6 ± 14.5 | 107.6 ± 18.8 |
| Slow wave sleep (min) | 205.9 ± 27.5 | 216.9 ± 18.8 | 215.4 ± 24.6 | 214.1 ± 12.4 | 227.5 ± 16.8 |
| Paradoxical sleep (min) | 39.8 ± 7.4 | 37.1 ± 6.8 | 35 ± 4.6 | 35.3 ± 5.2 | 24.9 ± 4.6*** |
| Sleep latency (min) | 26.3 ± 12.2 | 18.1 ± 6 | 19.8 ± 6.8 | 24 ± 11.3 | 12.3 ± 5.5* |
| PS latency (min) | 20 ± 5.7 | 26.7 ± 9.7* | 26.4 ± 10.7 | 31 ± 12.3* | 56.3 ± 15.5*** |
| Number of sleep cycles and PS episodes | 17.5 ± 2.1 | 17.8 ± 2.6 | 16.1 ± 3 | 16.3 ± 2.4 | 12.5 ± 2.2*** |
| Mean duration of cycles (min) | 10.9 ± 1.5 | 11.6 ± 1.8 | 11.7 ± 2.1 | 12.2 ± 1.7 | 12.7 ± 1.9 |
| Mean duration of PS episodes (min) | 2.4 ± 0.4 | 2.2 ± 0.3 | 2.3 ± 0.3 | 2.3 ± 0.3 | 2.1 ± 0.4 |

Values are mean ± SD ($n = 8$ for each group). Statistical evaluations were done with respect to controls. *$p < 0.05$; ***$p < 0.005$.

tude and a filter setting for half amplitude of low frequency (3 Hz) and for half amplitude of high frequency (30 Hz); the paper advanced at a rate of 6 mm/s.

Nisoxetine freshly dissolved in 0.9% NaCl (treated animals) or 0.9% NaCl (controls) was given to eight rats as the following treatments: a) NaCl or 0.05 mg/kg of nisoxetine; b) NaCl or 0.1 mg/kg of nisoxetine; c) NaCl or 0.25 mg/kg of nisoxetine; and d) NaCl or 1 mg/kg of nisoxetine. To prevent interference between different doses of nisoxetine, an interval of 1 week separated each treatment.

The tracings were visually scored according to the usual criteria for W, SWS, and PS by epochs of 20 s. The following sleep parameters were calculated: the total duration of sleep stages in minutes, their percentage, the latencies of sleep and PS, and the number and mean duration of sleep cycles and PS episodes.

Statistical estimation of differences was carried out by one-way analysis of variance (ANOVA) and post hoc comparison by paired two-tailed Student's *t*-test.

## RESULTS

As shown in Table 1, nisoxetine at 0.05 mg/kg significantly increased PS latency [$F(1, 7) = 11.2$, $p < 0.05$] without modifying the number of PS episodes. The mean duration and the amount of PS remained unchanged (Fig. 1). Nisoxetine also failed to change total sleep time and sleep organization (Table 1; Fig. 2).

In contrast to the result with lowest dose, after treatment with nisoxetine at 0.1 mg/kg, the percentage of PS significantly decreased [$F(1, 7) = 3.1$, $p < 0.05$, Fig. 1], although total sleep time was not modified (Table 1). After this dose, the percentage of SWS significantly increased [$F(1, 7) = 3.1$, $p < 0.05$, Fig. 2]. The other parameters remained unchanged (Table 1).

Nisoxetine at 0.25 mg/kg significantly increased only the PS latency [$F(1, 7) = 9.4$, $p < 0.05$, Table 1, Figs. 1, 2]. The other sleep variables remained unchanged (Table 1).

The effects of nisoxetine at 1 mg/kg concerned mainly PS. Indeed, PS appeared later [$F(1, 7) = 53.2$, $p < 0.005$, Table 1] and was scarce (PS%: $F(1, 7) = 57.1$, $p < 0.005$, Fig. 1; and PS time: $F(1, 7) = 37.7$, $p < 0.005$, Table 1], reflecting a reduction in the number of episodes [$F(1, 7) = 65.3$, $p < 0.005$]. All these changes were highly significant in comparison with controls. Although sleep latency significantly diminished [$F(1, 7) = 6.8$, $p < 0.05$], total sleep time did not change (Table 1). Finally, the number of sleep cycles decreased [$F(1, 7) = 65.3$, $p < 0.005$] without modification of their mean duration (Table 1). The percentage of SWS increased [$F(1, 7) = 57.1$, $p < 0.005$, Fig. 2]. All these changes were also significant in comparison with controls.

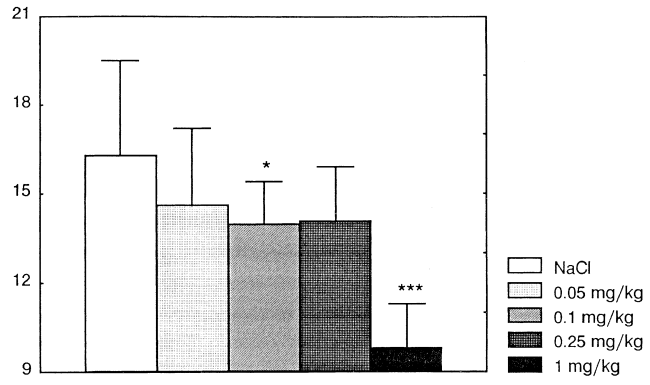


FIG. 1. PS percentage relative to total sleep time during the 6 h of recording after treatment with nisoxetine. The statistical evaluations were done with respect to controls. Mean ± SD. *$p < 0.05$; ***$p < 0.005$.

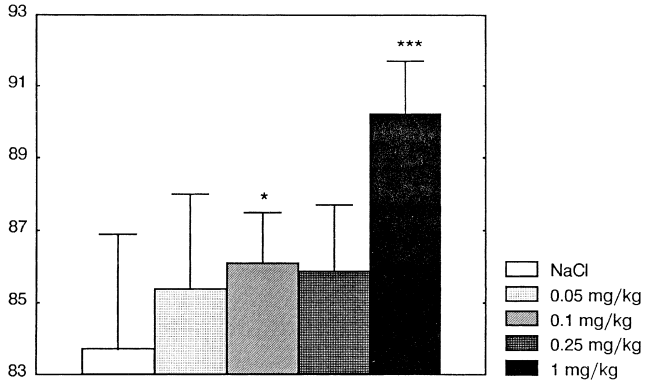


FIG. 2. SWS percentage relative to total sleep time during the 6 h of recording after treatment with nisoxetine. The statistical evaluations were done with respect to controls. Mean ± SD. *$p < 0.05$; ***$p < 0.005$.

## DISCUSSION

The involvement of NA systems in the regulation of PS, already shown in humans and animals by numerous authors (8,14,17,18), was confirmed here by the observed reduction in this stage without alteration in total sleep time. Although there was no significant change in the amount of SWS, the increase of its percentage after the high dose of nisoxetine was mainly due to the decrease of PS.

In this study, except for animals treated with 0.1 mg/kg of nisoxetine, a delayed appearance of PS was observed. Moreover, with the highest dose of nisoxetine, the amount of PS decreased, reflecting a diminution of the number of PS episodes without a change of their mean duration. Altogether, these results seem to confirm the permissive role of NA in the induction of PS (22).

As mentioned earlier, nisoxetine is a specific ligand of NA reuptake sites (27,29). Autoradiographic studies in animals have shown that high concentrations of binding sites for nisoxetine were found in both the locus coeruleus (LC) and the dorsal raphe nucleus (DR) (2,24). The LC and the DR are two nuclei, containing NA and serotonin (5-HT) neurons, respectively, critically implicated in PS regulation (10,21). Indeed, electrophysiological studies in animals have shown that NA neurons of the LC and 5-HT neurons of the DR decreased firing during SWS and ceased firing during PS (9,16). Furthermore, it has been shown that PS was increased when NA neurons stopped their discharge after in vivo cooling of the LC (1). Therefore, nisoxetine could probably delay PS by activating NA and, indirectly, 5-HT systems.

Classical antidepressants are usually mixed monoamine reuptake blockers, which are often effective on NA and 5-HT systems (13). Because a short PS latency and an enhanced number of rapid eye movements during PS seem characteristic in depressive patients (7,15,19), their effects on sleep have been extensively studied in humans and animals. The primary common effect of these compounds is their ability to reduce PS (4,12,14,20). Another group of antidepressants, the alpha-2-adrenoceptor antagonists (e.g., idaxozan and mirtazapine) also diminish PS (6,26).

The action of this presumed potential antidepressant (28) and its effects on PS in rats seem to be in agreement with the noradrenergic and serotonergic hypothesis of depression (15) and the short PS latency observed in depressive patients (7,15,19).

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