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Flurbiprofen in rapid eye movement sleep deprivation induced hyperalgesia

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HIGHLIGHTS

- REM sleep deprivation (REMSD) resulted in reduced pain thresholds in the rat.
- Flurbiprofen is a non-steroid anti-inflammatory agent with neuroprotective effects.
- Flurbiprofen was used for the first time in REMSD induced hyperalgesia.
- Flurbiprofen provided improvement against painful stimulation in REMSD rats.

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ABSTRACT

Background: Rapid eye movement (REM) sleep deprivation induces hyperalgesia in healthy rats. Here, we evaluated the effects of flurbiprofen, an anti-inflammatory and neuroprotective agent, on the increased thermal responses observed in REM sleep deprived rats.
Methods: Forty female rats were divided into four groups following 96-hour REM sleep deprivation: intraperitoneal injections of placebo, and flurbiprofen 5 mg/kg, 15 mg/kg and 40 mg/kg were made in CONT ($n = 10$), FBP5, FBP15 and FBP40 groups respectively. Pain threshold measurements were performed three times at baseline (0.hour), at the end of REM sleep deprivation (96.hour) and at 1 h after injections (97.hour) by hot plate and tail-flick tests.
Results: REM sleep deprivation induced a significant decrease in pain thresholds of all rats (hotplate: 0.hour vs 96.hour, 9.75 ± 2.85 vs 5.10 ± 2.02, $p < 0.001$; tail flick: 0.hour vs 96.hour, 11.92 ± 4.62 vs 7.92 ± 5.15, $p < 0.001$). Flurbiprofen in 15 mg/kg and 40 mg/kg doses significantly improved pain tolerance measured by tail flick test (tail flick in FBP15 and FBP40 groups: 96.hour vs 97.hour, 7.01 ± 4.97 vs 8.34 ± 3.61 and 5.06 ± 1.57 vs 7.04 ± 2.49, $p < 0.05$ for both).
Conclusion: 96 h of REM sleep deprivation resulted in reduced pain thresholds in both hot plate and tail flick tests. Flurbiprofen was used for the first time in a rat model of REM sleep deprivation, and it provided anti-nociceptive effects in 15 mg/kg and 40 mg/kg doses. Flurbiprofen may have the potential for treatment of painful syndromes accompanying insomnia or sleep loss.

1. Introduction

The effect of rapid eye movement sleep (REM) deprivation on pain perception in healthy rats was first studied by Hicks et al. [1]. They demonstrated that 24-, 48- and 72-hour REM sleep deprivation resulted in increased pain response to painful electrical stimuli [1]. In a subsequent study, Hicks et al. [2] showed that reduced pain threshold was prolonged up to 96 h after the termination of 4 days of REM sleep deprivation. Similarly, Onen et al. [3] reported that 48- and 72-hour REM sleep deprivation reduced the vocalization thresholds (increased pain sensitivity) to mechanical noxious stimulus in rats. In an attempt to further clarify the hyperalgesic effects of REM sleep deprivation, Onen et al. [4] used four different types of noxious stimuli, *i.e.* mechanical, thermal, electrical and chemical, and found a significant increase in the behavioral responses of rats. Taken together, these data clearly show that REM sleep deprivation induces a kind of hyperalgesia in healthy rats.

There may be several explanations for nociceptive effects of REM sleep deprivation. In a study, mechanical sensitivity was compared between nerve-injured animals and sleep-deprived healthy animals, and common spinal mechanisms including nitric oxide and metabotropic

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glutamate receptor 5 were suggested for both nerve-injury and sleep-deprivation related hyperalgesia [5]. Several studies investigated the relationship of opioidergic activity and REM sleep deprivation [6–8]. Ninety-six hours of REM sleep deprivation abolished analgesia induced by morphine, phosphoramidon and cold water [6]. Furthermore, potentiation of opioidergic anti-nociception by monoamines was prevented by REM sleep deprivation in rats [7]. These data suggested that REM sleep deprivation altered pain-inhibitory processes mediated by opioidergic and monoaminergic pathways. In a more recent study, reduced opioidergic neurotransmission in the brain as the potential reason for REM sleep deprivation induced hyperalgesia was investigated by autoradiographic mapping of receptor binding [8]. Authors stated that μ-opioid receptor binding was unaltered following REM sleep deprivation or during the recovery period [8].

Although the exact mechanism of action is not clear, some chemicals or drugs were tested to reduce the algesic effects of REM sleep deprivation. In 2009, Damasceno et al. [9] administered intraperitoneal amitriptyline, a tricyclic anti-depressant, for eleven days in a rat model of REM sleep deprivation. They failed to find any analgesic effect of amitriptyline in 72- and 96-hour sleep deprived rats [9]. In a more recent study, WIN55,212-2, a cannabinoid substance, was applied to 96-hour REM sleep deprived rats [10], and prevented pain behavior in response to hot plate pain model. Non-steroidal anti-inflammatory drugs are also well-known analgesic agents. Recently, flurbiprofen was shown to have neuroprotective effects besides its anti-inflammatory and analgesic properties in a model of brain ischemia [11]. Accordingly, we evaluated the effects of flurbiprofen on the hyperalgesia induced by REM sleep deprivation. Our hypothesis was that flurbiprofen modulates pain perception in rats subjected to 96 h of REM sleep deprivation.

2. Materials and methods

2.1. Animals

Forty female Sprague–Dawley rats (aging 8–10 weeks, weighing 135–175 g) were subjected to 96 h of REM sleep deprivation. All animals were supplied by Trakya University Experimental Animals Unit. They were kept under standard conditions (22 ± 1 °C; 12/12 h light–dark cycle; food and water access *ad libitum*). Every rat was used only once. They were sacrificed shortly after the test to minimize the suffering of the animal by exsanguination after high-dose general anesthesia. All the protocols in this study were approved by the local committee of ethics. Animals were divided into four groups: CONT, placebo control; FBP5, flurbiprofen 5 mg/kg i.p.; FBP15, flurbiprofen 15 mg/kg i.p.; and FBP40, flurbiprofen 40 mg/kg i.p.

2.2. REM sleep deprivation protocol

We used a well-known modified multiple flower pot technique to induce REM sleep deprivation [12]. In this model, several rats are deprived together in order to prevent isolation stress. We placed 5 rats into one cage containing 6 small platforms (6 cm in diameter) surrounded by water (2–3 cm height). One platform was kept empty to reduce immobilization stress. Thus, the animals were able to freely move and interact with each other. REM sleep is associated with muscle atonia, and when an animal enters REM sleep it falls from the platform into the water. Thus, the animal wakes up and climbs onto platform again. This method prevents REM sleep and permits other sleep stages.

2.3. Flurbiprofen administration

Flurbiprofen was purchased (FLURBIFEN 10 mg tab., Bilim Pharmaceuticals, Turkey). The drug tablets were ground in a metal garlic press and dissolved in normal saline (0.9% NaCl solution). Intraperitoneal injection volumes were a 0.5 ml single-dose for all groups. The CONT group received placebo in 0.5 ml saline. FBP5, FBP15 and FBP40 groups received 5 mg/kg, 15 mg/kg and 40 mg/kg flurbiprofen in 0.5 ml injection volume, respectively. These doses of flurbiprofen were shown to be neuroprotective [11,13].

2.4. Hot plate test

Individual animals were placed on a hot plate at baseline, at the end of 96-hour REMSD and 1 h after drug administration. The temperature of the hot plate (Ugo Basile, Biological Research Apparatus Company, Comerio, Italy) was maintained at 52 ± 1 °C. The time to withdraw a hindpaw, lick of a hindpaw or jumping off to avoid thermal nociception was measured in seconds. And the animal was immediately removed from the hot plate at that time point. A time period of 20 s was used as the cut-off time for a rat that did not respond to avoid tissue damage that may result from excess heat. All measurements were conducted between 08:00 and 10:00 h.

2.5. Tail-flick test

In order to better identify the pain thresholds of rats, we also used a tail-flick test as the second pain test. After the completion of all hot-plate measurements, all rats were subjected to the tail-flick test (Commat, Ankara, Turkey) during 10:00–11:00 h. The tail-flick device has a photocell system which measures working time automatically. The latency to the first sign of a rapid tail-flick was taken as the behavioral end point. To prevent tissue damage, we established a 20 s cut-off time. Tail flick assays were made for three times: at 0.hour (beginning of sleep deprivation), 96.hour (end of sleep deprivation) and 97.hour (1 h after FBP injection).

2.6. Statistical analysis

All data were given as means and standard deviations unless otherwise indicated. Normal distribution of variates was tested by a Kolmogorov Smirnov test. The difference in the percent change of pain perception between the study groups were tested by Kruskal Wallis ANOVA and *post hoc* Dunn's method. Intergroup comparisons were made by ANOVA and *post hoc* Bonferroni tests. Intragroup comparisons of repeated measures were made by repeated measures ANOVA. A p value lower than 0.05 was accepted as statistically significant.

3. Results

All animals survived after 96 h of REM sleep deprivation, but their body weights decreased approximately by 7% (Table 1). There was no significant difference between body weight changes of the groups. Pain threshold changes during 96-hour REMSD and after flurbiprofen administration are given in Fig. 1. REMSD induced a significant decrease in pain thresholds of the rats measured by both hotplate (Fig. 1a) and tail-flick (Fig. 1b) tests in all study groups. The mean decreases in pain thresholds of CONT, FBP5, FBP15 and FBP40 groups were 49%, 46%, 50% and 46% respectively with the hot plate test ($p < 0.001$); and 20%, 35%, 40% and 45% respectively with the tail-flick test ($p < 0.001$). At

Table 1
Body weight measurements in study groups before and after 96 h of REM sleep deprivation (REMSD). There was no significant difference between the study groups in terms of percent change during the REMSD protocol.

	Baseline weight, g	Last weight, g	Percent change*
CONT (n = 10)	161.20 ± 7.36	150.50 ± 8.48	0.07
FBP5 (n = 10)	163.50 ± 8.51	148.20 ± 7.43	0.10
FBP15 (n = 10)	165.20 ± 5.59	154.00 ± 7.16	0.07
FBP40 (n = 10)	166.70 ± 4.83	155.20 ± 5.59	0.07

Abbreviations: CONT, placebo control; FBP5, flurbiprofen 5 mg/kg group; FBP15, flurbiprofen 15 mg/kg group; FBP40, flurbiprofen 40 mg/kg group.
* P > 0.05, ANOVA.