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The H1 histamine receptor blocker, chlorpheniramine, completely prevents the increase in REM sleep induced by immobilization stress in rats

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ABSTRACT

Chlorpheniramine is a selective antagonist of the H1 histaminergic receptor subtype and its effects in humans include somnolence. Chlorpheniramine affects sleep in rats, mainly by decreasing REM sleep. On the other hand, stress by immobilization induces an important increase in the percentage of REM sleep. In this study we analyzed the effects of blocking histaminergic receptors on REM sleep induced by immobilization stress. Adult male Wistar rats were chronically implanted for sleep recording. Immobilization stress was induced by placing the rat in a small cylinder for 2 h. Experimental conditions were: A. Control; B. Stress; C. Stress plus vehicle and D. Stress plus chlorpheniramine. Independent experiments were done both in the dark, as well as the light period. Results showed that the increase in REM sleep observed after immobilization stress was completely abolished by chlorpheniramine, both in the dark and in the light phase. Furthermore, the decrease in REM sleep was significant even when compared to the non-stressed control rats. REM sleep latency was also significantly longer during both light phases. The present results suggest that REM sleep is quite sensitive to histaminergic blockage. It is possible that chlorpheniramine is also blocking the cholinergic mechanisms generating REM sleep.

1. Introduction

The histaminergic neurons are mainly located in the tuberomammillary nucleus, from where they widely project to several areas of the central nervous system (Lin, 2000). Histamine receptors have been classified into three subtypes (H1, H2 and H3) according to their pharmacological characteristics. Both the H1 and the H2 histaminergic receptor subtypes are located postsynaptically, while the H3 subtype is located in both the pre- and postsynaptic membrane (for review see: Hill et al., 1997). Chlorpheniramine (CPA) is a well known antagonist of the H1 histamine receptor subtype (Gutkowski et al., 1985). CPA was among the first available antihistamines and has been widely used because of its effectiveness as an antiallergenic agent (Gutkowski et al., 1985). CPA readily crosses the blood brain barrier and one of its collateral effects is the induction of sedation (Bassano and Caille, 1979; Boyle et al., 2006). In fact, this sedative effect forced the industry to develop a second generation of non-sedative antihistamines (Slater, 1999; Mann et al., 2000). However, the effects of CPA on sleep have been controversial and the sedative effect is not always observed (Karamanakos et al., 2004; Karamanakos, 2007). Nevertheless, a clear inhibition of the REM sleep stage has been reported systematically after administration of CPA (Wauquier and Niemegeers, 1981; Saitou et al., 1999). Moreover, this effect has been observed in several species, including humans (Nicholson et al., 2004).

On the other hand, growing evidence indicates a close relationship between the stress response and the sleep pattern (Bodosi et al., 2000; Bonnet et al., 2000; Zhang et al., 1988). One of the most conspicuous features of this relationship is the induction of REM sleep after 2 h of stress by immobilization (Rampin et al., 1991). This notable increment in REM sleep implies the participation of a number of neurotransmitter systems. Studies using neurotoxin DSP-4 have indicated a mediation of noradrenergic neurons (Gonzalez et al., 1995). Voltametric analysis of the serotonergic neurons in the raphe nucleus has shown that there is an activation after immobilization stress (Cespuglio et al., 1995; Houdouin et al. 1991). Furthermore, studies using naltrexone have indicated that the opioidergic system is also involved in the increase in REM sleep after immobilization stress (Vazquez-Palacios et al., 2004).

Histaminergic neurons located in the tuberomamilar nucleus have been involved in the stress response (Endou et al., 2001; Watanabe and Yanai, 2001). Thus, it is possible that the histaminergic system also participates in the induction of REM sleep by stress. In the present study, CPA, an H1 histaminic receptor blocker that commonly

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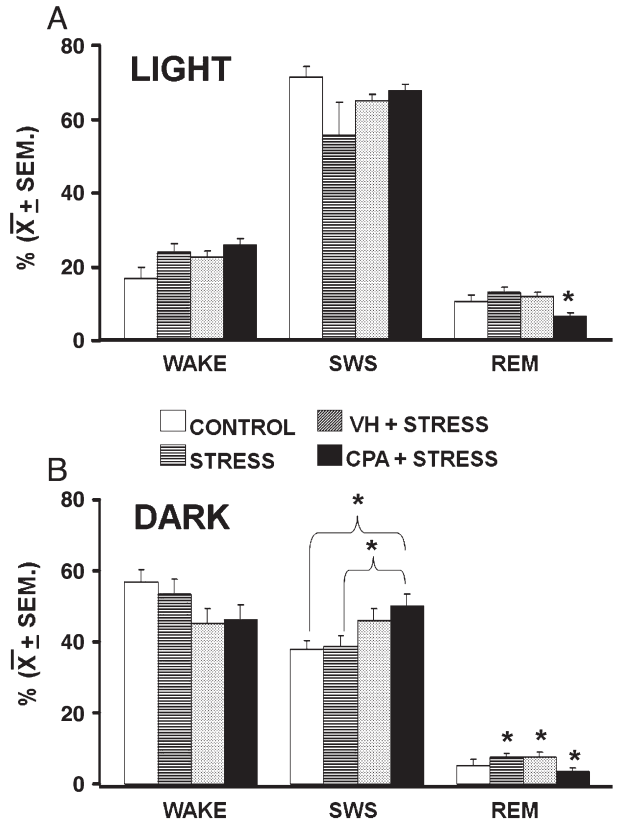

Fig. 1. Percentage of sleep stages in 8 h of polygraphic recordings after 2 h of stress by immobilization. Panel A: recording during the light period. Panel B: recording during the dark period. Control: no manipulation; stress: 2 h of immobilization; VH-stress: 2-hour immobilization stress plus 0.2 ml vehicle IP; CPA-stress: 2-hour immobilization stress plus 20 mg/kg bw chlorpheniramine IP. *$p<0.05$ compared to control.

suppresses naturally occurring REM sleep, was administered to prevent the increase in REM induced by immobilization in rats.

2. Methods

Adult male Wistar rats (350–400 g bw), bred in our own vivarium were used. Animals were housed in standard vivarium conditions and under 12/12 normal light/dark cycle (lights on 9:00 AM) with food and water available *ad libitum*. All procedures were in accordance to the *Guide for the Care and Use of Laboratory Animals* of the NIH. Under deep general anesthesia induced with a cocktail (ketamine: 3.75 mg/100 g; xylazine: 0.19 mg/100 g and acepromazine: 0.038 mg/100 g ip), rats were chronically implanted with a standard set of electrodes for sleep recording. After a two-week recovery period, the rats were habituated to the experimental cages for 24 h.

EEG and EMG were recorded for 8 h (9:00–17:00 h). Recordings were scored in a blind fashion and following previously published criteria (Takeuchi, 1970). Immobilization stress was achieved by placing the animal inside a plexiglass cylinder during the 2 h prior to the lights turned on. Recordings were obtained after the following conditions:

A. Control: ($N=14$)
B. Stress: After 2 h of stress by immobilization, without any injection. ($N=10$)
C. Stress plus the administration of vehicle. ($N=8$)
D. Stress plus CPA (chlorpheniramine maleate (Sigma) 20 mg/kg bw) dissolved in an ethanol/water solution. Doses were injected IP at the end of the stress period and in a 0.2 volume ml. ($N=9$)

The same procedure was repeated with the other rats during the dark phase of the cycle. Therefore, the stress was applied during the last 2 h of the light–dark cycle and recordings started when the lights went off. The number of subjects in the dark period groups was:

E. Control ($N=13$)
F. Stress ($N=10$)
G. Stress plus vehicle ($N=10$)
H. Stress plus CPA ($N=10$)

2.1. Statistical analysis

Data were analyzed using the Kruskal–Wallis ANOVA followed, when significant, by Kruskal–Wallis post-hoc test between pairs to find the source of significance.

3. Results

Fig. 1 shows the percentages of the different sleep stages. During the light phase of the cycle (Panel A), both wakefulness and SWS did not show any significant modifications in any of the conditions tested. REM sleep showed a trend to increase in the stress (B) and stress plus vehicle group (C). The stressed group treated with CPA (D) showed a significant decrease compared to the control group. During the dark phase of the light–dark cycle (Panel B) the stressed group treated with vehicle and with CPA showed a trend to decrease that did not reach statistical significance. SWS showed an increase that reached statistical significance in the stressed groups treated with CPA (H). Concerning REM sleep, a significant increase was observed in the stressed group (F) and in the stressed group treated with vehicle (G). The stressed group treated with CPA, however, showed a significant decrease even when compared with control.

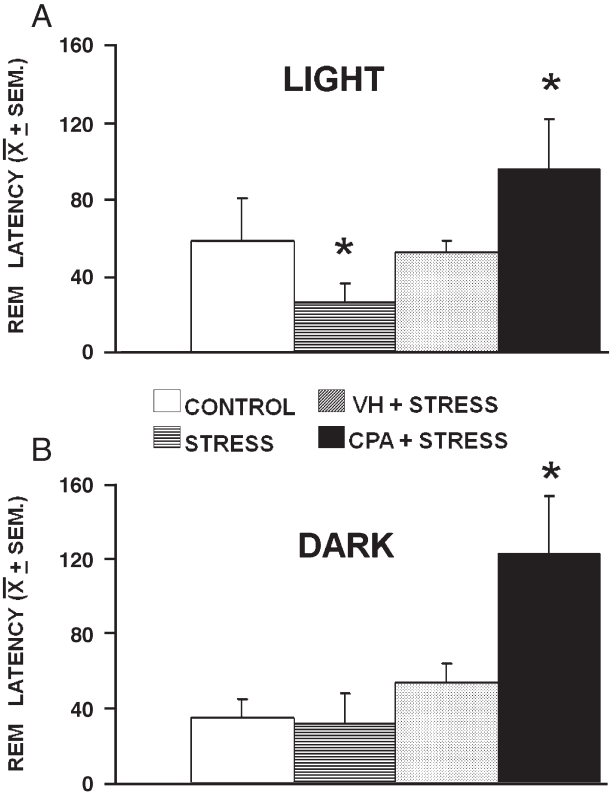

Fig. 2. Latencies to REM sleep after a 2-hour immobilization stress. Panel A: recording during the light period. Panel B: recording during the dark period. Control: no manipulation; stress: 2-hour immobilization stress; VH-stress: 2-hour immobilization stress plus 0.2 ml vehicle IP; CPA-stress: 2-hour immobilization stress plus 20 mg/kg bw chlorpheniramine IP. *$p<0.05$ compared to control.

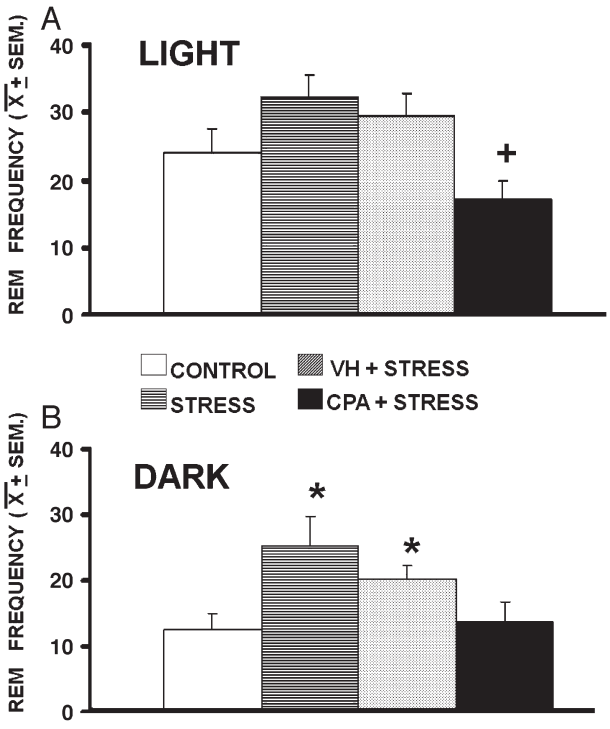

Fig. 3. Frequency of REM sleep bouts in 8 h of polygraphic recordings after a 2-hour immobilization stress. Panel A: recording during the light period. Panel B: recording during the dark period. Control: no manipulation; stress: 2-hour immobilization stress; VH-stress: 2-hour immobilization stress plus 0.2 ml vehicle IP; CPA-stress: 2-hour immobilization stress plus 20 mg/kg bw chlorpheniramine IP. *$p<0.05$ compared to control. +$p<0.05$ compared to all the groups.

REM sleep latencies are displayed in Fig. 2. A significant increase in REM latency is observed in the stressed group treated with CPA when compared to the control group. This effect was observed in both phases of the light–dark cycle.

Fig. 3 shows the analysis of REM sleep bouts frequency. During the light phase of the light–dark cycle (upper panel) there was an increase in REM frequency due to stress, however, it did not reach statistical significance. When CPA was administered to stressed rats, REM sleep frequency decreased to even lower values than those of the control group. During the dark phase of the light–dark cycle (lower panel), stressed groups showed a significant increase in REM sleep frequency compared to controls. When CPA was administered to stressed rats, the frequency in REM sleep returns to control values.

4. Discussion

Ever since the pioneering studies of Rampin et al. (1991), the notable increase in REM sleep after stress in rats has been repeatedly corroborated (Palma et al., 2000). Some determinants of the effect have been characterized as the nature of the stress (Velazquez-Moctezuma et al., 1993), the duration of the stress (Marinesco et al., 1999; Bonnet et al., 2000), the influence of the circadian cycle (Koehl et al., 2002), among others. Although the rats spent the 2 h of stress mostly awake, the increase in REM sleep after immobilization is not due to sleep deprivation. Rampin et al. (1991) have compared immobilization with sleep deprivation for 2 h and the increase in REM sleep is absent in the sleep deprived rats. Concerning the participation of different neurotransmitter systems, some reports indicate the participation of the noradrenergic system (Gonzalez et al., 1995), of the serotonergic system (Sinha, 2006; Haleem et al., 2007) and even of the opioidergic system (Vázquez-Palacios et al., 2004).

On the other hand, the participation of the histaminergic system in the sleep–wake cycle has been a matter of controversy for years. Vanni-Mercier et al. (2003) have recorded histaminergic neurons located in the tuberomammillary system that display maximum activity during wakefulness; it decreases during SWS and are almost silent during REM sleep. Histamine H1 receptor antagonists including mepyramine, diphenhydramine, and chlorpheniramine reduce waking and REMS and increase SWS in rats and/or dogs (Monti et al., 1986; Wauquier et al., 1981). However, the only systematic result obtained after the administration of antihistamines that block the H1 receptor is a clear reduction of REM sleep (Wauquier et al., 1981; Saitou et al., 1999; Nicholson et al., 2004; Marzanatti et al., 1989). Furthermore, experiments in knockout mice lacking the H1 histaminergic receptor show no alteration of sleep features (Huang et al., 2006). Thus, although the histaminergic system is involved in REM sleep regulation, its mechanism of participation is not clear.

Moreover, the cholinergic regulation of REM sleep is well known. Stimulation of muscarinic receptors mainly at pontine level readily generates a remarkable increase in REM sleep (Velazquez-Moctezuma et al., 1990). It has been suggested that the M2 muscarinic receptors subtypes are responsible for this increase in REM sleep by pontine stimulation (Velazquez-Moctezuma et al., 1989, 1991) although systemic administration of M1 muscarinic blockers in rats suggests that the M1 muscarinic receptor subtype is also involved in REM sleep regulation (Zoltowski et al., 1993). In addition, it has been reported that H1 histamine receptor blockers, including chlorpheniramine, have a clear antimuscarinic action (Orzechowski et al., 2005). Thus, it is possible that blockade of muscarinic receptors by chlorpheniramine is the cause of the decrease observed in the percentage of REM sleep in stressed animals. Nevertheless, considering that histaminergic neurons fire during wakefulness and are silent during REM sleep, the inhibitory influence of histaminergic blockers on REM sleep could include the participation of more than one neurotransmitter system. The exact mechanism remains to be elucidated.

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