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# Effect of Alpha-Adrenoceptor Blockade on Sleep and Wakefulness in the Rat

JYRKI P MAKELA AND ILKKA T HILAKIVI[1]

*Department of Physiology, University of Helsinki, Siltavuorenpenger 20 J SF-00170 Helsinki, Finland*

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MAKELA, J P AND I T HILAKIVI *Effect of alpha-adrenoceptor blockade on sleep and wakefulness in the rat* PHARMACOL BIOCHEM BEHAV **24**(3) 613–616, 1986 —Three alpha-adrenoceptor antagonists with different subtype selectivity were administered IP at the beginning of the light period of the illumination cycle to rats whose sleep-wake pattern was subsequently recorded for 12 hours Yohimbine (1 mg/kg) initially increased active wakefulness but did not affect REM sleep Phentolamine (10 mg/kg) and prazosin (0 5 and 1 0 mg/kg) increased the amount of REM sleep during the latter half of the light period The mechanism of this delayed increase in REM sleep may be related to a greater extent of alpha-receptor binding as well as an optimal, moderate concentration of prazosin and phentolamine in the brain during the latter half of the light period

Yohimbine Phentolamine Prazosin Alpha-adrenoceptor Sleep Wakefulness Rat

RECENT experiments on rats [4, 7, 9, 14, 18, 21] and cats [10, 11, 12, 15, 19, 20] have demonstrated that alpha-adrenoceptors are involved in the regulation of sleep as well as wakefulness In both species activation of postsynaptic alpha-1 adrenoceptors appears to facilitate wakefulness [10,18], whereas their role in the regulation of sleep, and especially of REM sleep, is still somewhat controversial

In cats, phentolamine and prazosin, two alpha-adrenoceptor antagonists, were reported to facilitate REM sleep [11, 12, 15, 19] Phentolamine has generally been regarded as an equally potent antagonist of alpha-1 and alpha-2 receptors, and prazosin as a selective antagonist of alpha-1 receptors In rat brain, however, alpha-1 receptors may be composed of two subtypes [17] Prazosin, on the other hand, was recently reported to affect prejunctional alpha-2 receptors in rat peripheral nerves [24] Recently, prazosin was reported to inhibit REM sleep in rats at a wide dose range during the first half of the light period [18] The reasonably selective alpha-2 adrenoceptor antagonist, yohimbine, on the other hand, showed a biphasic effect on the amount of REM sleep in rats [7] In cats only its well-known activating effect was noticed at doses of 0 5–2 0 mg/kg [15] Since the alpha-receptor subtype selectivity of prazosin and phentolamine in the rat may differ from that in the cat in peripheral tissues [22], and possibly in the brain [23], we have in this study investigated the relationship between activation of alpha-receptors and regulation of sleep and wakefulness in rats, with a particular interest on REM sleep

## METHOD

Eight male rats of the Wistar strain, at the age of 3–4 months and at the body weight of 250–350 g, served as subjects Under chloral hydrate (approximately 300 mg/kg IP) and Xylocain-Adrenalin (20 mg/ml + 12 5 μg/ml) anesthesia two stainless steel screw electrodes were inserted to the skull, one anteriorly and one posteriorly, to record cortical electrogram A screw electrode was inserted into the dorsal hippocampus using the stereotaxic coordinates of 3 5 mm posterior to bregma, 3 1 mm lateral to the midline and 1 0–2 0 mm ventral to the dura Two coaxial (cardiac pacemaker) wires were sutured into the deep neck muscles

Recordings were started 1–2 weeks after surgery, and performed within 3 months (September–November) An interval of 1–2 weeks was allowed between drug recordings Rats were housed in individual cages (25×30×35 cm) in an ambient temperature of 22–24°C and a relative humidity of 50±5% An illumination cycle of 12 hr light/12 hr dark (light on at 06 00 hr) was used The rats were allowed ad lib food (Astra-Ewos diet) and water

The experiments were started at approximately 07 00 hr with IP injections of the alpha-blockers in a volume of 1 ml/kg dissolved with appropriate vehicle and diluted in distilled water, or with IP injection of a respective volume of 0 9% sodium chloride Recording conditions were the same as housing conditions during the light period Three rats were recorded simultaneously Continuous 12-hr neocortical EEG, hippocampal EEG, and nuchal EMG recordings were carried out using a Grass polygraph The paper speed was 10 mm/sec The time constant of 0 3, low pass filter of 60 Hz and gain of approximately 100 μV/cm were used in the EEG recordings

The paper records were visually classified into five stages of sleep and wakefulness *Active waking* (AW) was scored when the EEG showed low amplitude, fast frequency activity and the amplitude of the EMG was continuously high and

[1]Requests for reprints should be addressed to Ilkka T Hilakivi, M D

TABLE 1

THE EFFECT OF YOHIMBINE, PHENTOLAMINE AND PRAZOSIN ADMINISTRATION ON THE STAGES OF SLEEP AND WAKEFULNESS IN THE RAT

| | (N) | AW | DW | LSWS | DSWS | REM |
|---|---|---|---|---|---|---|
| control | (5) | 36 5 ± 2 4 | 11 1 ± 2 4 | 32 2 ± 4 2 | 12 5 ± 5 9 | 7 5 ± 2 4 |
| yohimbine 1 0 mg/kg | (5) | 36 6 ± 10 0 | 8 0 ± 2 0* | 35 4 ± 9 5 | 10 9 ± 1 8 | 9 0 ± 3 1 |
| phentol-amine 10 0 mg/kg | (5) | 31 8 ± 6 0 | 9 9 ± 2 8 | 32 3 ± 4 1 | 15 0 ± 6 1 | 10 9 ± 2 1* |
| prazosin 0 5 mg/kg | (5) | 30 9 ± 7 4 | 8 1 ± 1 7† | 32 1 ± 6 9 | 18 8 ± 4 5 | 10 1 ± 2 0† |
| prazosin 1 0 mg/kg | (5) | 34 6 ± 8 8 | 7 0 ± 1 0† | 27 1 ± 3 3† | 20 1 ± 7 1* | 11 2 ± 3 4* |

Mean percentages (±S D ) of 12-hr recording time, *=$p<0$ 05, †=$p<0$ 01 (paired $t$-test compared with control, two-tailed)

frequent movement deflections could be seen *Drowsy waking* (DW) was characterized by interference of synchronous slow wave activity (though less than 25% of the epoch) with low amplitude, fast frequency EEG in addition to the diminution of EMG amplitude and the lessening of movement deflections *Light slow wave sleep* (LSWS) was scored when 25–50% of the EEG tracing was composed of irregular high amplitude slow waves *Deep slow wave sleep* (DSWS) was scored when the amount of slow waves exceeded 50% of the epoch *REM sleep* (paradoxical sleep, REM) was characterized by low amplitude, mixed frequency activity in cortical EEG, synchronous high amplitude 5–8 Hz (theta) activity in hippocampal EEG, and very low amplitude activity in EMG with occasional short deflections due to muscle twitches

Each rat was recorded 4–5 times A balanced order of control and experimental recordings was used Each group consisted of 5 rats The drugs and doses were yohimbine hydrochloride 1 0 mg/kg (Boehringer Ingelheim), phentolamine mesylate 10 0 mg/kg (Regitine, Ciba-Geigy), and prazosin hydrochloride 1 0 mg/kg (Pfizer Inc ) Yohimbine was dissolved in distilled water, phentolamine mesylate solution (Regitin) was diluted with distilled water, and prazosin hydrochloride was dissolved in a small volume of heated 10% water solution of N,N-dimethylacetamide and diluted with water

The visually scored data were stored on disk memory of a computer for calculations and statistics Recordings after drug treatments were compared with recordings of the same rats after saline injections using paired, two-tailed $t$-test

## RESULTS

### *Yohimbine*

Yohimbine (1 0 mg/kg) initially increased active waking (during 1st hr from 42 2 to 52 0%, n s ) and decreased drowsiness The amount of REM sleep did not change (Table 1, Fig 1)

### *Phentolamine*

Pilot studies with phentolamine at doses of 5–40 mg/kg did not show any clear effects Phentolamine (10 0 mg/kg) increased the percentage of REM sleep between 4 and 12 hr of recordings This increment was also reflected in the statistics of total recording time (Table 1) Phentolamine did not lengthen REM episodes but increased their number from 34 2±12 1 to 49 4±16 5 ($p<0$ 05)

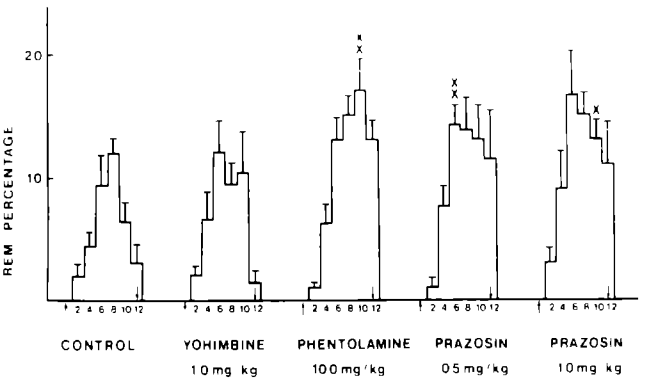


FIG 1 The effects of yohimbine, phentolamine, and prazosin on REM percentages (±S E M ) during six consecutive 2-hr periods of the 12-hr recording time Lights (arrow) were turned on 1 hr before IP drug injections (time 0 hr) and turned off at time 11 hr Each bar represents the mean of 5 rats The recordings after drug treatments as indicated were compared with recordings of the same rats after saline injections using a paired, two-tailed $t$-test *=$p<0$ 05 **=$p<0$ 01

### *Prazosin*

Prazosin (1 0 mg/kg) initially increased active waking time (1–2 hr 38 6 to 44 5%, n s ) and decreased REM sleep Thereafter percentages of REM sleep were increased during all the remaining five 2-hr periods (Fig 1) At the dose of 0 5 mg/kg active wakefulness increased from 38 4 to 47 4% (n s ) during the first 2 hours During the remaining 10 hours the

amount of drowsiness was decreased and the amount of REM sleep increased (Table 1) Prazosin 1 0 mg/kg increased the number of REM episodes during 12 hr from 27 8±8 1 to 47 8±9 5 ($p<0$ 01), with 0 5 mg/kg of prazosin the increase remained nonsignificant Neither dose of it changed the duration of REM episodes In total time statistics the decrease in drowsiness, increase in REM sleep, and the tendency to increased amount of DSWS were dose-related (Table 1)

## DISCUSSION

The alpha-2 adrenoceptor antagonist yohimbine was recently reported to facilitate REM sleep at doses as low as 0 03 mg/kg [7], and at doses up to 3 mg/kg to depress it for about 4 hours and thereafter to facilitate its occurrence [7] Its activating effect at doses between 1 and 3 mg/kg have been well documented in rats [7,18] and cats [15] In agreement with these studies a clear but short-lasting enhancement of active wakefulness at the cost of REM sleep, and more importantly, at the cost of drowsy waking was observed at 1 mg/kg of yohimbine, a dose which does not affect clearly rat locomotor activity [2,3] or only causes a slight degree of excitation [6]

In contrast to yohimbine, both phentolamine and prazosin, two alpha-adrenoceptor antagonists with affinity for the alpha-1 subtype, increased REM sleep In our pilot studies the doses of phentolamine (10 mg/kg) and prazosin (0 5–1 0 mg/kg) were chosen on the basis of our experience from cats [10, 11, 15, 19, 20] and on the basis of behavioral effects of these drugs [2, 3, 6] At these doses which are without clear acute effects on overt behavior of rats [2, 3, 6], an initial 2–3 hr depression of REM sleep was recorded This result is in partial agreement with the recently reported consistent decrease in REM sleep in rats during the first part of the light period after 0 125–1 mg/kg of prazosin [18] Our main finding, the increase in REM sleep only during the latter half of the light period, may be related to an increase in alpha-receptor binding in rat brain during this part of the illumination cycle [13] In this case the initial lack of effect on REM sleep could be due to hypotension [22] or hypothermia [5] Furthermore, cat, which does not show any clear circadian sleep-wake rhythm, responds to prazosin (0 5–1 0 mg/kg) and to phentolamine (5–10 mg/kg) with a prompt increase in REM sleep but at higher doses (prazosin 3–10 mg/kg, phentolamine 20 mg/kg) with an initial depression of REM sleep [11, 12, 15, 19]

Alternatively, alpha-1 receptor antagonists may act in rats as unspecific sedatives inhibiting motor activity which would favour sleep mechanisms as a secondary effect, especially at the beginning of the dark period, i e , in our study during the last 2 recording hours when the rats were spontaneously most active The lack of effect of prazosin (0 75–3 mg/kg SC) or phentolamine (10 mg/kg) in behavioral tests [2, 3, 6] argues against this alternative

The present results show that both phentolamine and prazosin favoured deep SWS and REM sleep at the cost of drowsy waking The tendency to a decreased amount of drowsiness and increased deep SWS during alpha-blockade might be related to the prolonged suppressing effect of IV or local administration of alpha-blockers on the firing rate of 5-HT neurons in the rat dorsal raphe nucleus [1] These single unit and microiontophoretic data tend to suggest that alpha-blockers might affect sleep by modulating serotoninergic transmission in the brain leading to increased deep SWS and REM sleep In dogs, however, opposite data were found after phentolamine (1 mg/kg × 6 subcutaneously) short-lasting arousals were increased and the number of REM episodes decreased [8]

The relationship between cerebral noradrenergic activity and REM sleep has long been a controversial issue Recent neuropharmacological studies in rats [7,14] and cats [10, 15, 20] have implicated a positive role for noradrenaline in the executive mechanisms of REM sleep, possibly through activation of beta-receptors in the brain [10,14] In rats, the rebound of REM sleep after its instrumental deprivation was eliminated by administration of phentolamine (5 mg/kg) or phenozybenzamine at the beginning of the rebound sleep [21], leading the authors to suggest that alpha-receptor activation contributes positively to the mechanisms of REM sleep This suggestion may, however, be premature since in this REM sleep rebound situation [21] a tendency to an increased amount of REM sleep after an initial 12-hr suppression was recorded Thus, it is possible that in this REM rebound study [21], as well as in our study with a two-fold dose of phentolamine (10 mg/kg), the amount of REMS may depend on a continuously increasing number of cerebral alpha-receptors [13] as well as a simultaneously decreasing concentration of alpha-blocker in the brain towards the latter half of the light period Thus, during the light period which coincides with the circadian peak in the number of alpha receptors in rat brain [13], a moderate or optimal level of alpha-receptor blockade may even facilitate REM sleep

We have recently suggested that in cats the balance between alpha- and beta-adrenoceptor activation in the brain may be crucial for facilitation by noradrenaline of either active wakefulness or REM sleep and concurrent inhibition of drowsiness or sedation [10] The present findings, together with alpha-receptor binding data [13], suggest that rats may not after all differ from cats in this respect, contrary to what was earlier suggested by us [16] and others [4,7], mainly based on experiments on rats showing ineffectiveness of yohimbine or phentolamine in counteracting clonidine These results are in sharp contrast to competitive antagonism observed in cats [15,20]

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