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The Effect of Alpha and Beta Adrenergic Receptor Blockers on Sleep in the Rat[1]

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HARTMANN, E. AND G. ZWILLING. *The effect of alpha and beta adrenergic receptor blockers on sleep in the rat.* PHARMAC. BIOCHEM. BEHAV. 5(2) 135–138, 1976. We investigated the effects of the beta adrenergic blocker, propranolol, and the alpha adrenergic blocker, phenoxybenzamine, on sleep patterns in the rat by means of multiple 8-hr and 24-hr polygraphic recordings. Propranolol had no clear effect on time spent in waking, synchronized sleep or desynchronized sleep. Phenoxybenzamine at a dose of 40 mg/kg produced a significant increase in desynchronized sleep time and in the number of desynchronized sleep periods. This is consistent with the view that there may be a mechanism inhibiting the onset of desynchronized sleep periods which involves norepinephrine acting at alpha adrenergic receptors in the brain.

Sleep D-sleep Desynchronized sleep Propranolol Phenoxybenzamine Adrenergic blockers

ONE surprising but consistent finding of recent sleep research is that drugs of many different classes: hypnotics, tranquilizers, antidepressants, stimulants, alcohol – in fact almost all drugs studied – affect sleep somewhat similarly, by decreasing desynchronized sleep time (D-time). Only a few drugs have been shown to produce an increase in D-time (reviewed in [10]); these are specifically drugs such as alpha-methylparatyrosine [14,21] and reserpine [7, 11, 19] which produce a decrease in brain catecholamine levels or activity. We have suggested that there is an inverse relationship between functional brain catecholamine levels and D-time, and that D-sleep may have a role in restoring catecholamine levels or the integrity of catecholamine systems [8,9]. Study of adrenergic or dopamine receptor blockers may elucidate what postsynaptic receptor mechanisms are involved in this relationship. In addition, there is a great deal of evidence suggesting that the catecholamines play a role in maintaining wakefulness and it would be important to know what receptor mechanisms might be involved.

Although it has not been conclusively proven that alpha and beta adrenergic receptors exist in the brain, considerable pharmacologic evidence exists that alpha and beta agonists and antagonists have differential effects on the hypothalamus at least, and probably other brain systems [1, 2, 5]. Evidence also favors the existence of dopamine receptors in the brain [3,4].

We have previously studied the dopamine receptor blocker, pimozide, in the rat [18], and chlorpromazine (CPZ) in long-term human sleep studies [12]. In both situations we found a slight decrease in waking and an increase in total sleep time (significant for CPZ but not for pimozide) but no change in D-time.

In the present study, we investigated the effects of an alpha-adrenergic blocker, phenoxybenzamine, and of a beta-adrenergic blocker, propranolol. Although these drugs are often considered representative of their classes, there are data indicating that both substances have further actions besides adrenergic blockade, including some antihistaminic effect and possible inhibition of re-uptake of the catecholamines [6].

The effects of alpha and beta blockers on sleep have not been previously reported in the rat. A preliminary study in the cat showed no clear-cut effects on sleep [20], while a recent study in man demonstrated that alpha adrenergic blocker, thymoxamine, produced an increase in D-time for a brief period of time, several hours after administration [25]. Portions of the present studies have been reported briefly at two meetings [13,16].

METHOD

A total of thirty male rats were used in these studies. These were Norwegian albino rats supplied by Charles River Laboratories at the age of 90–120 days (weight about 250 g); each rat was implanted with the usual cortical, hippocampal, and nuchal muscle electrodes for long-term sleep recordings. Rats recovered for 2 weeks prior to any recordings. Each rat was then adapted to the laboratory conditions for a further 2 week period. After this, groups of 5 to 10 rats took part in studies comparing placebo with each dose of propranolol and phenoxybenzamine (see Tables 1 and 2). Each rat had a number of 8-hr recordings on placebo or drugs usually at 96-hr or at 1-week intervals, in a balanced design. In a separate study, 6 rats had a series or 24-hr studies taking, in balanced order, placebo and

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phenoxybenzamine 40 mg/kg, the dose that seemed of greatest interest on the bases of the 8-hr recordings. On each experimental day, rats were fed at 9 a.m. either 10 g of wet mash (placebo) or 10 g of wet food mash containing one of the drugs. The rats were always slightly hungry on their normal feeding schedule, so that the placebo food or drug-containing food was always consumed within fifteen minutes. (This is our usual laboratory procedure and that of other laboratories using oral drug administration. Comparison of our values on this schedule with earlier data and with data of other laboratories using ad lib feeding showed little difference in sleep patterns). Records began at 9:30 a.m. and continued either until 5:30 p.m. or until 9:30 a.m. the next day. The illumination cycle was 12 hr light, 12 hr darkness. All records were scored blind by an experienced scorer; each 30-sec epoch was scored as waking (W), synchronized sleep (S), or as desynchronized sleep (D) according to the usual sleep laboratory criteria [23,24]. According to this criteria, W is characterized by low amplitude mixed cortical and hippocampal activity with high amplitude muscle; S by high amplitude, relatively slow cortical and hippocampal activity, moderately low muscle activity; D by low amplitude cortical activity, hippocampal theta, and very low muscle activity. Every tenth record was scored by an additional scorer for reliability. Five to 8 animals were studied at each drug dose originally, and further groups were studied at doses that seemed to produce changes.

RESULTS

Table 1 presents the effects of propranolol in doses of 1–40 mg/kg on various sleep parameters. Propranolol had no significant effect on W, S, or D-time over the dose range studied, nor did it significantly alter number of D-periods, (In this study number of D-periods refers to the total number of such periods scored regardless of separation; episodes of D-sleep separated by as little as one page – 30 seconds – of W or S are scored as separate D-periods), cycle length, or other variables studied. A few 24-hr studies were done which likewise showed no clear effects of propranolol.

Table 2 presents the effects of phenoxybenzamine. There were no significant effects at the lower doses but at 40 mg/kg D-time and the number of D-periods were increased. Effects in the same direction, not reaching significance, were found with 20 mg/kg and 80 mg/kg. The mean length of each D-period remained constant; there was no increase in brief interruptions in D-periods. Waking time was not significantly altered by the drug but waking tended to be low at lower doses with a gradual increase in waking time at higher doses. (Waking time consisted almost entirely of wakefulness after the first sleep onset; sleep latency was usually 10–30 min on drug and on placebo.) S-sleep was unchanged.

Results on 24-hr studies on 6 animals who received placebo as well as phenoxybenzamine 40 mg/kg are presented in Table 3. D-time and the number of D-periods was significantly increased during the second and third 8-hr and over the entire 24-hr. For the entire 24-hr period mean total D-time was 116 min on placebo, 146 min on phenoxybenzamine; mean number of D-periods was 64.8 on placebo, 89.8 on phenoxybenzamine. Both differences are significant ($p<0.05$).

There was no effect over the first few hours of recordings; the increase in D-time and in number of D-periods occurred chiefly at hours 3–6 and 12–18 (Fig.

TABLE 1

EFFECTS OF PROPRANOLOL ON SLEEP IN THE RAT (8-HR RECORDINGS)

	1 mg/kg+2 mg/kg (n = 7)	5 mg/kg (n = 6)	10 mg/kg (n = 6)	20 mg/kg (n = 17)	40 mg/kg (n = 7)
W	85.1% ± 5.3%	91.9% ± 14.8%	100.5% ± 5.8%	97.3% ± 5.6%	96.0% ± 8.5%
S	105.0% ± 5.4%	107.3% ± 10.9%	100.4% ± 5.1%	99.4% ± 4.3%	101.9% ± 5.2%
D	126.0% ± 13.8%	99.9% ± 18.9%	104.7% ± 8.4%	110.4% ± 9.6%	91.7% ± 11.2%

Drug values expressed as percent of mean placebo values ± S.E.M. for the same groups of animals. Mean placebo value is the mean of usually three placebo recordings. All values were first calculated in minutes, and then expressed as percentages.

No values were significantly different from placebo.

TABLE 2

EFFECTS OF PHENOXYBENZAMINE ON SLEEP IN THE RAT (8-HR RECORDINGS)

	2 mg/kg + 5 mg/kg (n = 5)	10 mg/kg (n = 8)	20 mg/kg (n = 9)	40 mg/kg (n = 18)	80 mg/kg (n = 6)
W	84.8% ± 11.5%	91.1% ± 6.9%	91.8% ± 4.8%	93.5% ± 4.2%	105.9% ± 11.7%
S	109.7% ± 6.3%	102.0% ± 3.3%	102.3% ± 5.6%	100.1% ± 2.8%	92.0% ± 8.8%
D	96.2% ± 16.9%	113.2% ± 7.1%	110.8% ± 23.5%	128.3% ± 17.9%*	113.1% ± 21.1%
Number of D-periods	87.9% ± 13.4%	115.5% ± 12.8%	109.5% ± 11.0%	132.1% ± 8.5%*	97.9% ± 13.0%

Drug values expressed as percent of mean placebo values ± SEM for the same groups of animals.

*$p<0.05$, two-tailed, t-test for correlated samples.

TABLE 3

EFFECTS OF PHENOXYBENZAMINE ON SLEEP IN THE RAT (24-HR RECORDINGS)

	First 8-hr	Second 8-hr	Third 8-hr	24-hr
W	107.1% ± 8.1%	91.1% ± 7.5%	84.0% ± 13.5%	93.8% ± 6.0%
S	98.8% ± 5.3%	103.3% ± 6.4%	105.2% ± 5.7%	102.3% ± 2.4%
D	73.7% ± 21.6%	127.3% ± 11.0%*	177.1% ± 31.5%*	124.8% ± 14.6%*
Number of D-periods	126.3% ± 19.4%	137.5% ± 20.3%*	143.5% ± 29.5%*	138.5% ± 14.8%*

Drug values expressed as percent of mean placebo values ± S.E.M. for the same groups of 6 animals.
*$p<0.05$, two tailed, t-Tests for correlated samples.

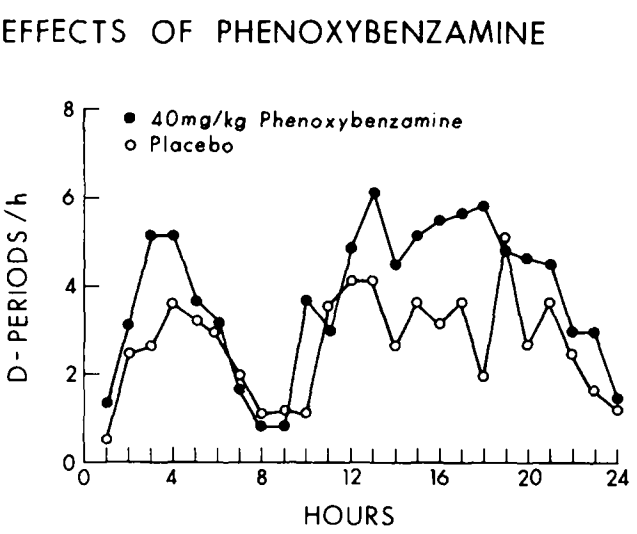

FIG. 1. Each point represents the mean of 6 animals.

1). The intervening hours (6 - 12) are evening hours when the rats eat and spend most of their time awake.

DISCUSSION

Propranolol apparently had no effect either on sleep-waking distribution, nor on distribution of synchronized or desynchronized sleep states. The decreased W and increased D at the lowest doses almost reached statistical significance but the fact that a slightly higher dose (5 mg/kg) produced absolutely no effect on a similar number of animals makes it unlikely that these changes are meaningful. In conjunction with the similar negative findings of a study in the cat [20], this makes it probable that beta adrenergic receptors are not directly involved in regulating sleep mechanisms, at least in these species. It is of interest that in man drowsiness and increased sleep is frequently reported after the clinical use of propranolol; this has yet to be investigated fully, but in a small scale sleep laboratory study of patients receiving high doses of propranolol, we were unable to demonstrate significant changes in sleep length or sleep patterns (unpublished).

Phenoxybenzamine does appear to have an effect on sleep - an increase in desynchronized sleep and a decrease in waking found at 40 mg/kg and to a small extent at 20 and 80 mg/kg. The fact that this effect is more prominent at 40 than at 80 mg/kg is not entirely surprising: Waking time (Table 2), and number of awakenings increased gradually with increasing dose of phenoxybenzamine, and were considerably increased at 80 mg/kg, indicating probably a peripheral side effect of the drug, resulting in mildly disturbed sleep. We have noted in a large number of situations, both in man and in the rat, that such sleep-disturbing effects are associated with increased waking but even more with a decrease in D-time, so that this D-reducing effect at the highest dose would counteract any tendency to an increase produced by the drug. We have noted similar results, for instance, with alpha-methyl-paratyrosine (AMPT) in the rat: The maximal increase in D-time is found at 75 mg/kg, while doses of 100 mg/kg produce a smaller increase and 200 mg/kg, approaching toxic levels, produced a decrease [14].

At 40 mg/kg, phenoxybenzamine does appear to produce an increase in desynchronized sleep, and an increase in number of D-periods, as well as a slight decrease in waking time. The magnitude of the overall change in D-time (30%) is not large, but is of vary similar magnitude to the increases in D-time we have demonstrated with AMPT and with 6-hydroxydopamine [14,17] in the rat and with reserpine in man [11]. Larger percentage changes can be seen if only the hours of peak effect are considered. The 40% increase in number of D-periods over the entire 24 hours (Table 3) is larger than we have seen with any other pharmacologic agent. It is not clear why an increase in measures of D-time occurred during the 8-hr recordings, but occurred only some hours later in the 24-hr recordings. The animals on 24-hr recordings did sleep somewhat differently the first 8-hr, with more waking time, possibly related to differences in the adaptation schedule.

We cannot be absolutely certain that central rather than peripheral effects are involved in the effects of phenoxybenzamine. Peripheral adrenergic blocking could produce side effects which could indirectly affect sleep or waking. However, such effects can often be picked up behaviorally and/or by examining waking time and the number of awakenings: A side effect disturbing to the animal results in more waking time and more awakenings during sleep accompanied by a relative decrease in D-time. As mentioned, our data suggest that such effects may have been present at the highest dose (80 mg/kg) of phenoxybenzamine. In addition, as mentioned, phenoxybenzamine has a number of effects aside from alpha-adrenergic blocking, so that one cannot be certain that the present results on sleep are related to alpha blocking. However, in view of similar results in man [25] with a different alpha blocker, thymoxamine, this would appear to be the most reasonable explanation.

Since phenoxybenzamine produced effects on D-time while propranolol, and pimozide [18] had no effect, these results suggest that the inverse relationship we have previously demonstrated between catecholamine levels or activity in the brain and D-time most likely depends on norepinephrine acting at alpha-adrenergic receptors. We have demonstrated that in the rat phenoxybenzamine reverses the D-sleep reducing effects of d- and l-amphetamines, which again suggests that an alpha-adrenergic mechanism may be involved [15]. The anatomic locus for these receptors is of course not certain, but it could be quite widespread in the brain, at noradrenergic endings in the cortex, for instance, or in the limbic system, the hypothalamus or the brain stem. Post-synaptic neurons from such areas may inhibit the brain stem centers involved in the initiation of D-sleep. Several centers have been suggested for this role; there is considerable evidence favoring the giant cells of the tegmental fields [22]. According to this postulated scheme, the alpha-adrenergic blocker reduces the discharge in these widespread post-synaptic neurons which normally have a tonic inhibitory action on the D-initiating areas of the brain stem. This tonic influence is reduced, making the initiation of D-periods easier, and thus increasing especially the number of D-periods. According to our views of the functions of sleep [9], the ensuing D-periods may then play a role in the restoration of the norepinephrine neurons or synapses.

Our hypothesis is that in some sense the norepinephrine reducing drugs such as AMPT, or perhaps an alpha-adrenergic blocker such as phenoxybenzamine, can be seen as an exaggeration or a parody of what happens during a normal waking period. Waking may consist of a reduction of functional norepinephrine levels or norepinephrine activity, perhaps by synaptic alterations which make the synapse less efficient. This results in less postsynaptic activity, and a reduction of the inhibition of D-periods. Desynchronized sleep then occurs which helps in restoring the norepinephrine systems.

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