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Possible Involvement of α_2-Adrenoceptor on the Hypnotic Action of Flunitrazepam

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DE SAINT HILAIRE, Z. AND J.-M. GAILLARD. *Possible involvement of α_2-adrenoceptor on the hypnotic action of flunitrazepam.* PHARMACOL BIOCHEM BEHAV **53**(1) 1–4, 1996.—This study examined whether the pharmacologic manipulation of catecholaminergic systems would affect the hypnotic action of flunitrazepam in rats. Flunitrazepam, a potent hypnotic, was used alone or in combination with α-methyl paratyrosine (αMPT), an inhibitor of the synthesis of catecholamines, and clonidine (CLN), an α_2-adrenoceptor agonist. Flunitrazepam significantly increased the amount of slow-wave sleep and the latency of paradoxical sleep (PS) and decreased the amount of PS. Administration of flunitrazepam to αMPT-treated rats significantly increased the number of sleep cycles and PS episodes as compared with flunitrazepam alone. Clonidine decreased total sleep time and significantly decreased PS. The association of flunitrazepam with CLN induced a decrease in PS and waking as compared with flunitrazepam alone. The possible involvement of noradrenergic mechanisms in modulating the effect of flunitrazepam on the rat sleep-waking cycle is proposed.

Sleep Benzodiazepine αMPT Clonidine Rat

IT HAS been indicated that the anxiolytic and sedative effects of the benzodiazepines compounds may be related to central serotonin (5-HT) and catecholamine (CA) turnover, respectively (6,7,15,18).

Benzodiazepines have been found to decrease the turnover of noradrenaline (NA) and dopamine (DA) in the brain (7,15). In rats, giving diazepam resulted in the accumulation of NA and DA in the hypothalamus, pons, medulla, midbrain, and striatum with reduction of their metabolites. In humans, benzodiazepine withdrawal is also associated with an increase of cathecholamine synthesis and turnover (12,19).

Locus coeruleus, a major noradrenergic nucleus in the brain, may have a functional role in the regulation of anxiety as well as vigilance states (1,4,14,23). Benzodiazepines have been found to decrease the firing of noradrenergic neurons in the locus coeruleus (5). The suppression of noradrenergic unit activity in the locus coeruleus could be due to the anxiolytic and sedative action of theses agents. In rats, synergistic interactions between benzodiazepines and α_2-adrenergic agonists has also been reported (20,21).

Despite their clinical use, the mechanisms underlying the actions of benzodiazepines upon sleep remain obscure. Although it is likely that GABA-ergic mechanisms underlie the effects of benzodiazepines on sleep, other systems may be implicated (2,8). However, we have previously shown that 5-HT mechanisms are probably not involved in the hypnotic action of a benzodiazepine such flunitrazepam (3).

In this context we investigated the possible involvement of noradrenergic mechanisms on the hypnogenic action of flunitrazepam combined with α-methyl paratyrosine (αMPT), an inhibitor of cathecholamine synthesis, and clonidine (CLN), an α_2-adrenoceptor agonist.

METHOD

We used male Wistar rats, weighing 250–280 g, in these experiments. They were implanted in the neck muscles with four electrodes for electroencephalogram (EEG) recordings and two electrodes for electromyogram (EMG), under pentobarbital (55 mg/kg) anesthesia.

After surgery, the animals were housed in individual recording cages under a constant temperature of 25°C and humidity of 55%, and with free access to food and water. They were then allowed 2 weeks for recovery and habituation to the experimental conditions. The daylight period was maintained between 0800 and 2000 h.

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The following experimental designs were used: a) physiologic saline solution (0.9%); b) flunitrazepam, 2 mg/kg alone; c) αMPT, 150 mg/kg alone; d) a combination of αMPT and flunitrazepam; 150 mg/kg αMPT was given 30 min before flunitrazepam; e) clonidine, 0.08 mg/kg alone; f) a combination of clonidine and flunitrazepam; 0.08 mg/kg clonidine was given 5 min before 2 mg/kg flunitrazepam.

Flunitrazepam, αMPT, and clonidine were used only at one dose corresponding to optimal effects on sleep variables (3,9,10,11,17). The treatments were given intraperitoneally in random order at 0800 h. To prevent interference between drugs, for each session involving combinations of flunitrazepam, we used different rats.

Sleep recording began at 0800 h and lasted for 12 h. Three different stages of EEG activity were visually scored into 20-s epochs: waking (W), slow-wave sleep (SWS), and paradoxical sleep (PS). Waking was characterized by rapid 20ν30 cycles per second (c/s) EEG activity of low amplitude and muscle activity; SWS by slow, 1–5 c/s EEG waves and small bursts of 10ν14 c/s rhythms called spindles; and PS by fast waves of low amplitude on the anterior part of the cortex, whereas the EEG activity of the posterior part was dominated by a diffusion of the highly rhythmic τ activity of the hippocampus. In addition, PS was characterized by an abolition of muscle tone.

We analyzed 11 sleep variables (Table 1). In addition, the cumulated occurences of each state of EEG activity were counted by 10-min epochs of total sleep time for PS and total recording time for W; their temporal evolution was expressed by level, slope, and curvature. All data were statistically analysed by using analysis of variance (ANOVA) followed by posthoc unpaired Student's *t*-test.

RESULTS

Effects of Flunitrazepam

Table 1 shows the effects of flunitrazepam on rat sleep. Total sleep time and SWS were significantly increased with 2 mg/kg flunitrazepam. Waking time and paradoxical sleep significantly decreased. The latency to the first PS episode was increased, and the total duration of PS was significantly less than in the controls (Fig. 1). Significant changes in the other PS variables were mixed: The number of PS episodes decreased and the average of PS episode duration increased.

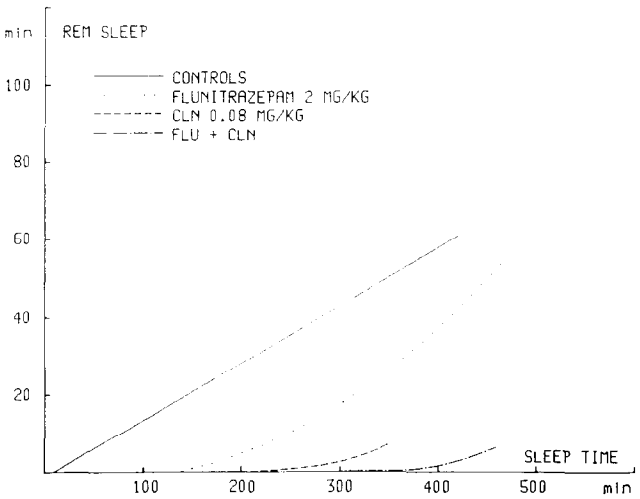

FIG. 1. General trend of PS in the rat under the effect of saline (—), 2 mg/kg flunitrazepam (...), 0.08 mg/kg CLN (---), and the combined administration of flunitrazepam and CLN (-.-.-).

Flunitrazepam also significantly increased the mean duration of the sleep cycle.

Effects of αMPT and Clonidine

At a dose of 150 mg/kg, αMPT had no effect on different sleep variables, except a tendency to increase PS. At a dose of 0.08 mg/kg, CLN decreased total sleep time during the first 4-h period of EEG recording and significantly decreased PS. The general trend of waking showed a significant increase (slope: $p < 0.05$) in comparison with controls (Fig. 2). Similarly, the latency from sleep onset to the first occurrence of PS was markedly lengthened. However, as shown elsewhere (10,17), the reduction of PS was proportional to the decrease of total sleep (Table 1). Also, the average of PS episode duration decreased significantly. Finally, the number of awakenings and stages shifts was significantly higher than in controls.

TABLE 1

SLEEP VARIABLES AFTER FLUNITRAZEPAM AND ITS COMBINATION WITH α-METHYL PARATYROSINE AND CLONIDINE

Sleep Variables (Min)	Controls (n = 16)	Flunitrazepam 2 mg/kg (n = 7)	α-MPT 150 mg/kg (n = 5)	CLN 0.08 mg/kg (n = 10)	α-MPT + Flunitrazepam (n = 6)	CLN + Flunitrazepam (n = 6)
Total sleep	441 ± 51	489 ± 19†	476 ± 19	404 ± 76	501 ± 53	501 ± 44††
Total waking time	208 ± 50	169 ± 17†	184 ± 20	256 ± 76	159 ± 53	159 ± 44††
Slow-wave sleep	376 ± 54	432 ± 23‡	387 ± 17	389 ± 76	434 ± 51	487 ± 46††
Paradoxical sleep	68 ± 12	57 ± 6†	89 ± 10	15 ± 11‡	67 ± 21	14 ± 9#
Sleep latency	12 ± 10	4 ± 3*	24 ± 16	9 ± 4	7 ± 11	2 ± 1††
PS latency	15 ± 9	157 ± 37‡	26 ± 22	305 ± 82‡	125 ± 27¶	385 ± 77**
No. of sleep cycles and PS episodes	35 ± 5	23 ± 2†	45 ± 6	11 ± 7‡	30 ± 2¶	11 ± 7#
Mean duration of sleep cycles	11 ± 2	13 ± 5†	9.3 ± 1	7 ± 1†	12 ± 1§	8 ± 1#
Mean duration of PS episodes	2 ± 0.3	3 ± 0.1†	2.1 ± 0.1	1.3 ± 0.4†	2.3 ± 0.6	1.3 ± 0.3#
No. of awakenings	127 ± 33	124 ± 5*	168 ± 24	209 ± 40‡	136 ± 39	151 ± 43††

Effects of flunitrazepam alone or combined with α-MPT and clonidine on sleep in the rat. Comparison with controls: *$p < 0.05$, †$p < 0.001$; ‡$p < 0.005$. Comparison of combined treatment with α-MPT: §$p < 0.01$; ¶$p < 0.005$. Comparison of combined treatment with flunitrazepam: #$p < 0.05$; **$p < 0.001$. Comparison of combined treatment with CLN: #$p < 0.05$; ††$p < 0.005$.

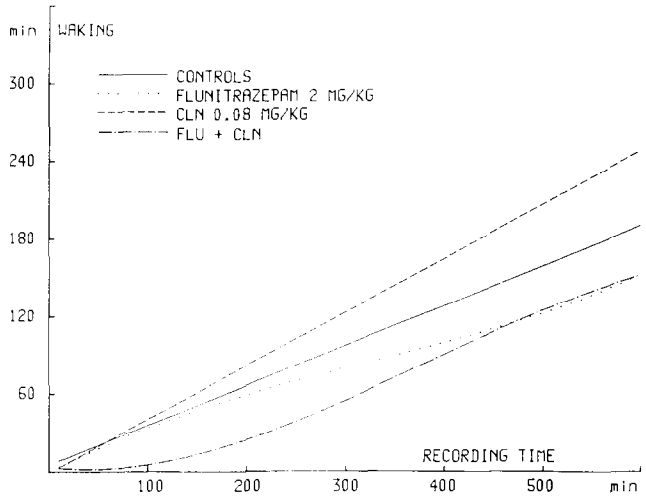

FIG. 2. General trend of waking in the rat under the effect of saline (—), 2 mg/kg flunitrazepam (...), 0.08 mg/kg CLN (---), and the combined administration of flunitrazepam and CLN (-.-.-).

Effects of Flunitrazepam in Association With αMPT and Clonidine

As can be seen in Table 1, after pretreatment with αMPT (150 mg/kg), flunitrazepam significantly increased the number of sleep cycles and PS episodes compared with flunitrazepam alone.

Flunitrazepam reversed the arousing effect of CLN: There was an increase of SWS and a decrease in the number of awakenings compared with CLN alone. The latency to the first occurrence of SWS was shorter than after flunitrazepam or CLN alone. In fact, the combination of CLN with flunitrazepam (Fig. 2) modified the evolution of waking by depressing its production, resulting in a significant level ($p < 0.001$) of curve compared with flunitrazepam and CLN alone.

Flunitrazepam did not antagonize the decrease of PS as was seen with CLN (Fig. 1); the other PS variables remained unchanged. The latency to the first PS episode was increased by the combined treatment (Table 1). The other sleep variables remained unchanged.

DISCUSSION

The present study shows that flunitrazepam produces several modifications in the normal architecture of sleep. Our results also confirm the hypnotic action of flunitrazepam in normal adult rats (3,8).

α-Methylparatyrosine is often used as an inhibitor of catecholamine synthesis in the CNS. It has been reported that 150 mg/kg αMPT decreases dopamine and noradrenaline fluorescence in the rat brain (9). After such a dose, the depletion of brain CA is only minimal; in particular, the synaptic transmission in CA synapses is only slightly impaired. Thus, this level of inhibition of CA synthesis seems to interfere with the action of flunitrazepam. The decrease of PS episodes by flunitrazepam was reversed after the inhibition of CA synthesis. This suggests that a limited decrease of synthesis of NA or DA is able to antagonize the effect of flunitrazepam on PS.

CLN is a very potent α_2-adrenoceptor agonist in the CNS, and its hypotensive action is clearly established. Previous studies have shown that the hypotensive effect of CLN is enhanced after benzodiazepine administration (12,22). In addition, the increased effect of CLN in reducing NA turnover during benzodiazepine withdrawal suggests an increase of α_2-adrenoceptor sensitivity (18). The increase of anxiolytic effect of benzodiazepine by α_2-adrenergic agonists is compatible with the hypothesis that changes in central NA transmission are involved in this activity of benzodiazepines (6,13,19).

Examination of the effects of the combined treatment of flunitrazepam and clonidine on sleep variables shows a potentiation of the effects of flunitrazepam alone. This was also the case for the general trend of PS (Fig. 1), which showed an accumulated decrease induced by these two drugs. However, the general trend of waking (Fig. 2) seems to indicate that the combined treatment did more than just accumulate the effects of the two drugs. The general trend of waking under the combined treatment was similar to that observed in other experiments with a dose of CLN two to four times higher (4,10,17). This cannot be due to the effect of flunitrazepam alone. In the first 200 min of recording time, the decrease of waking under the combined treatment was much more important than that induced by flunitrazepam alone, whereas the effect of flunitrazepam alone in this period was insignificant. Thus, CLN seems to potentiate the effects of flunitrazepam on the inhibition of waking. The low production of PS in our experiments suggests that the combined administration of CLN and flunitrazepam resulted in more than the simple addition of the effects of the two drugs alone. The slope of the curve indicated that the effect of flunitrazepam was potentiated by CLN. This is in favor of the GABA-ergic modulation of noradrenergic systems, which is in line with some effects observed clinically between this type of agonist of α_2-adrenoceptor and the ligands of the benzodiazepine receptors (16,20,21). However, the mechanisms by which benzodiazepine enhance GABA-ergic systems are not yet firmly established. The modulation of noradrenergic effects, which does correlate with the anxiolytic and sedative properties of flunitrazepam, may be an additional mechanism by which some benzodiazepines produce their effects, and could explain differences in the profile of activity of the benzodiazepine compound.

We propose a possible involvement of noradrenergic mechanisms in modulating the effect of flunitrazepam on the rat sleep-waking cycle.

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