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Effects of a D_2 Receptor Agonist RO 41-9067 Alone and With Clonidine on Sleep Parameters in the Rat

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PYTHON, A., Z. DE SAINT HILAIRE AND J. M. GAILLARD. *Effects of a D_2 receptor agonist RO 41-9067 alone and with clonidine on sleep parameters in the rat.* PHARMACOL BIOCHEM BEHAV **53**(2) 291-296, 1996.—The effects of RO 41-9067, a D_2 dopamine receptors agonist, on different sleep parameters were studied in the rat. RO 41-9067 dose dependently decreased paradoxical sleep, and only at the higher dose increased waking during the light period. In contrast, the higher dose of RO 41-9067 increased paradoxical sleep and decreased waking during the dark period. Finally, the combination of RO 41-9067 and clonidine significantly prevent the decrease of total sleep time and paradoxical sleep found after clonidine alone. These results, compared with those of classical D_2 dopamine receptors agonists, suggest an action for RO 41-9067 on D_2 dopamine receptors depending on the cerebral structure, a different action particularly on the striatum and/or on the structures responsible for paradoxical sleep. An active role for D_2 dopamine receptors and an interaction between noradrenergic and dopaminergic systems in the regulation of sleep is proposed.

Paradoxical sleep D_2 dopamine receptors α_2 Adrenoceptors RO 41-9067 Clonidine Rat

THE INFLUENCE of dopamine (DA) on sleep parameters is an unresolved question. Dopamine is thought to play a role in behavioral activation and sleep in particular paradoxical sleep (PS).

Pharmacological studies in humans and animals with D_2 dopamine receptor agonists have shown a dose-dependant biphasic effect on PS occurrence, low doses increasing, whereas high doses decreasing PS (3,4,14,22). On the other hand, intraventricular injection of small doses of DA increased PS and decreased locomotor activity in the rat (6). A study in narcoleptic dogs has also shown that D_2 dopamine receptors are involved in the regulation of cataplexy, a pathologic manifestation of PS atonia in narcolepsy (20).

In the light of these results, it can be hypothetized that inhibition of dopaminergic systems is involved in cortical activity and in PS atonia. Dopaminergic systems would promote behavioral waking and affect PS (8). In other words, inhibition of those systems via D_2 presynaptic receptors would be necessary for PS maintenance.

RO 41-9067, [(−)-2,3,4a,5,6,10b-hexahydro-7-hydroxy-2-methylbenzo (f)-quinolinone-4(^{1}H)-ethanol], is a selective dopamine autoreceptor agonist of the D_2 type (10,11). Studies with in vivo microdialysis conducted by Scherchlicht et al. in 1992, indicate that extracellular DA levels in the striatum are dose dependently decreased following acute RO 41-9067 administration in cats. They also reported a decrease of waking (W) and an increase of PS. Low doses of RO 41-9067 is believed to stimulate presynaptic receptors and cause a central dopaminergic depression, whereas high doses induce a central dopaminergic stimulation via both presynaptic and postsynaptic receptors. Contrary to classical neuroleptics, RO 41-9067 does not lead to catalepsy for doses that decrease DA liberation. The pharmacological profile of RO 41-9067 allows to study the contribution of DA in the regulation of W and PS stages.

Clonidine, an α_2 adrenoceptor agonist, causes, at low doses, a decrerase of PS by reducing adrenergic activity in the rat (13). In this case, clonidine may act on α_2 presynaptic adrenoceptors and inhibits noradrenaline discharge (23).

Based on the above considerations, the present study was designed to investigate the effects of RO 41-9067 on different sleep parameters.

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Then, because of the interaction between central dopaminergic and noradrenergic systems (1,7,12), a second series of experiment was also designed to see whether the combination of RO 41-9067 and clonidine will affect sleep parameters in the rat.

METHOD

Sixteen male Wistar rats, weighing 260–300 g, were used. Under Nembutal anesthesia (55 mg/kg, IP), they were implanted with three electrodes from frontal to occipital positions for electroencephalogram (EEG) as well as two electrodes for electromyogram (EMG) recording from the neck muscles. Two weeks after surgery the rats were placed in the recording cages (one rat/cage) for 1 week of habituation to the experimental conditions. Light period between 0800 and 2000 h, ambient temperature of 25°C, and humidity of 55% were maintained. Animals had free access to food and water. All recordings began at 0800 h and lasted for 24 h. The EEG recording appliance was Grass model 78D, with a 50 mV amplitude, a filter setting for half amplitude of low frequency: 3 Hz and for half amplitude of high frequency: 30 Hz and the paper progressed at the rate of 6 mm/s.

Three different stages of vigilance were visually scored for each 20-s epoch: waking (W), slow wave sleep (SWS), and paradoxical sleep (PS). W was caracterized by rapid cortical activity (20–30 cycles/s), and intense motor activity; SWS by slow EEG waves (one to five cycles/s) and burst of 10–14 cycles/s (spindles); PS by rapid EEG activity (20–30 cycles/s) with low amplitude, rhythmic theta activity (6–7 cycles/s), and an abolition of muscle tone.

In the first part of the experiment (Tables 1 and 2, Fig. 1), a group of eight rats were recorded after intraperitoneal (IP) injection of: a) saline (NaCl 0.9%); and b) RO 41-9067: 0.03, 0.3, 0.6, and 3 mg/kg (dissolved in NaCl 0.9 %).

All treatments were given at 0800 h To avoid interferences between different doses, at least 1-week intervals separated each experimental session.

In the second part of the experiment (Tables 3 and 4, Fig. 2), a second group of eight rats were recorded after IP treatment with: a) saline (NaCl 0.9%); b) 0.005 mg/kg of clonidine (dissolved in NaCl 0.9%); and c) RO 41-9067 (0.03 mg/kg) was given 10 min after clonidine (0.005 mg/kg).

The results obtained with the combination (RO 41-9067 and clonidine) were compared to those obtained with RO 41-9067 (0.03 mg/kg) or and clonidine alone.

Eleven different sleep parameters were calculated (see tables). W% were calculated in respect to controls, SWS% and PS in respect to total sleep time.

For the statistical evaluation of Experiment 1, all the parameters were tested by one-way analysis of variance (ANOVA), post hoc comparison by Scheffe and Student's *t*-tests. A multifactor ANOVA, have been used for Experiment 2.

RESULTS

Effects of RO 41-9067 Alone During the Light Period

The effects of RO 41-9067 4 h after its administrations are shown in Table 1.

For 0.3, 0.6, and 3 mg/kg, the amount of PS decreased and its latency increased (Table 1). PS% in respect to total sleep time decreased for all the doses tested (Table 1). The diminution of PS in amount and percent for 0.3 and 3 mg/kg reflected the decrease of the number of PS episodes without modification of their mean duration, whereas for 0.6 mg/kg, an augmentation of the mean duration of PS episodes was induced (Table 1). The SWS% in respect to total sleep time increased for all the doses tested (Table 1).

RO 41-9067, at the dose of 0.3, 0.6, and 3 mg/kg, decreased the number of sleep cycles while their mean duration increased (Table 1). The higher dose of 3 mg/kg, decreased total sleep time (Table 1). Finally, during the light period, the number of stage shift and the number of awakenings decreased for 0.3, 0.6, and 3 mg/kg (Table 1).

Effects of RO 41-9067 Alone During the Dark Period

Following 0.3 mg/kg, the number of awakenings increased (Table 2).

For the dose of 0.6 mg/kg, the number of awakenings and

TABLE 1

EFFECTS OF RO 41-9067 ON SLEEP PARAMETERS DURING THE FIRST 4 HOURS OF THE LIGHT PERIOD (0800–1200 h)

Variables	NaCl $n = 8$	0.03 mg/kg $n = 8$	0.3 mg/kg $n = 8$	0.6 mg/kg $n = 8$	3 mg/kg $n = 7$
Total recording (min)	240	240	240	240	240
Total sleep (min)	163 ± 11.4	164.5 ± 15.5	175.6 ± 13.8	161.7 ± 24.3	70.5 ± 44.8*
W (min)	77 ± 11.4	75.5 ± 15.5	64.4 ± 13.8	78.3 ± 24.3	169.5 ± 44.8*
SWS (min)	139.2 ± 10.4	144.9 ± 13.1	157.5 ± 12†	147 ± 21.7	70.1 ± 43.9‡
PS (min)	23.8 ± 4.6	19.6 ± 3.3	18.1 ± 3*	14.7 ± 4*	0.4 ± 1.13*
W%	32.1 ± 4.7	31.4 ± 6.5	26.8 ± 5.7	32.6 ± 10.1	70.6 ± 18.7*
SWS%	85.4 ± 2.6	88.1 ± 1.4†	89.8 ± 1.3*	91 ± 1.8*	99.7 ± 0.8*
PS%	14.6 ± 2.6	11.9 ± 1.4†	10.2 ± 1.3*	9 ± 1.8*	0.3 ± 0.8*
Sleep latency (min)	21.7 ± 3.8	17.1 ± 5.4	20 ± 12.4	35.7 ± 31.6	47.6 ± 41.3
PS latency (min)	15.4 ± 9.9	23.6 ± 5.3	39.2 ± 11*	59.5 ± 16.4*	142.9 ± 41.7*
Number of cycles and PS episodes	12 ± 2.1	9.6 ± 2.6	8.3 ± 2.1*	5.5 ± 1.6*	0.3 ± 0.8*
Mean duration of cycles (min)	11 ± 1.1	14.2 ± 3.3	15.9 ± 3.2*	20.4 ± 5.7*	13.2 ± 1.7*
Mean duration of PS episodes (min)	2 ± 0.2	2.1 ± 0.3	2.3 ± 0.6	2.8 ± 0.6†	2.1 ± 0.4
Number of stage changes	114.1 ± 22.7	106.4 ± 30.5	88.5 ± 24†	67.4 ± 27.3*	38.1 ± 30‡
Number of awakenings	50.4 ± 11.4	47.9 ± 15	39.3 ± 12.8†	30 ± 13.7*	19.6 ± 15†

Drugs were given by IP route. The statistical evaluations were done with respect to NaCl. Mean ± SD.
*$p < 0.005$; †$p < 0.05$; ‡$p < 0.01$.

TABLE 2

EFFECTS OF RO 41-9067 ON SLEEP PARAMETERS DURING THE 12 HOURS OF THE DARK PERIOD (2000-0800 h)

Variables	NaCl $n = 8$	0.03 mg/kg $n = 8$	0.3 mg/kg $n = 8$	0.6 mg/kg $n = 8$	3 mg/kg $n = 7$
Total recording (min)	720	720	720	720	720
Total sleep (min)	212.3 ± 31.6	207.3 ± 44.8	200.3 ± 44.5	221.3 ± 33	314.8 ± 68.2*
W (min)	507.7 ± 31.5	512.6 ± 44.7	519.7 ± 44.5	498.7 ± 33	405.2 ± 68.2*
SWS (min)	190.7 ± 21.6	184.5 ± 37.5	180.5 ± 36.8	196.3 ± 25.8	263.1 ± 52.3*
PS (min)	21.6 ± 11.5	22.8 ± 9	19.8 ± 8.7	25 ± 9.1	51.7 ± 23.4*
W%	70.5 ± 4.4	71.2 ± 6.2	72.2 ± 6.2	69.3 ± 4.6	56.3 ± 9.5*
SWS%	90.3 ± 4.4	89.4 ± 2.9	90.6 ± 3	89 ± 3.2	84 ± 5.4*
PS%	9.7 ± 4.36	10.6 ± 2.9	9.4 ± 3	11 ± 3.2	16 ± 5.4*
Sleep latency (min)	24 ± 21.5	37.5 ± 34	30.3 ± 26.6	33 ± 31.7	10 ± 12.6†
PS latency (min)	10 ± 5	11.1 ± 9	24 ± 17.8	11.3 ± 6.2	12.5 ± 10.5
Number of cycles and PS episodes	14.9 ± 9.1	13.9 ± 6.1	13.5 ± 6.1	15.6 ± 5.1	32 ± 12.7*
Mean duration of cycles (min)	9 ± 2.3	9.4 ± 2.1	8.1 ± 1.4	8.4 ± 1.3	7.8 ± 1.5
Mean duration of PS episodes (min)	1.6 ± 0.2	1.7 ± 0.2	1.5 ± 0.3	1.7 ± 0.2	1.7 ± 0.2
Number of stage changes	262.3 ± 25.7	262 ± 36.2	300 ± 34.1	308.8 ± 45.5*	409.1 ± 68.1*
Number of awakenings	123 ± 11.3	123.6 ± 15.9	143 ± 18.8*	146.1 ± 21.5*	185.9 ± 38.4*

Drugs were given by IP route. The statistical evaluations were done with respect to NaCl. Mean ± SD.
*$p < 0.05$; †$p < 0.005$; ‡$p < 0.01$.

the number of stage changes increased during the twelve hours of the recording (Table 2).

The augmentation of PS (amount and %) after RO 41-9067 (3 mg/kg) was related to the increase of the number of PS episodes (Table 2). With the high dose of 3 mg/kg, the percent of SWS decreased during this period (Table 2). Finally, with the dose of 3 mg/kg, sleep latency decreased and total sleep time increased (Table 2). So, whereas the number of awakenings increased, the W decreased after administration of 3 mg/kg of RO 41-9067 (Table 2).

Effects of Clonidine Alone During the Light Period

As shown in Table 3, clonidine affect various sleep parameters in the rat.

Immediately after its administration, clonidine induced bursts of large amplitude waves (400-600 mV; frequency: 6 Hz; mean duration: 1-5 s). During this period, the rats remained motionless.

PS decreased in time and percent (Fig. 2), reflecting a diminution of the number of PS episodes and of their mean duration. PS latency and SWS% in respect to total sleep time increased (Table 3).

The diminution of the number of sleep cycles and their mean duration during the first 4 h resulted in an decrease of total sleep time. Sleep latency was also decreased. Finally, the number of awakenings increased during this period (Table 3).

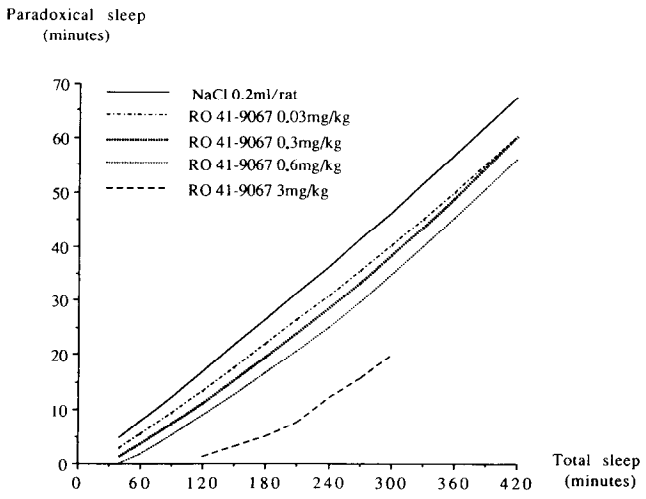

FIG. 1. Effect of RO 41-9067 on the general trend of paradoxical sleep during the light period.

Effects of Clonidine Alone During the Dark Period

Total sleep time, SWS, and PS increased in time during this period and percent during the first 6 h of EEG recording. There was also an increase in the number of PS episodes. In contrast, SWS% in respect to total slep time decreased during this period (Table 4).

Relative to the augmentation of the number of sleep cycles without modification of their mean duration, total sleep time increased (Table 4). The number of stage changes and the number of awakenings increased (Table 4).

Effects of the Combination of RO 41-9067 and Clonidine During the Light Period

Effects of the combination of RO 41-9067 and clonidine compared with those of RO 41-9067 alone. In this experiment, the combination of RO 41-9067 and clonidine was compared to the results of RO 41-9067 alone of the first experiment.

The combined treatment decreased total sleep time and increased waking when compared to RO 41-9067 alone (Table 3). Both the number of sleep cycles and their mean duration were also decreased (Table 3).

The amount of PS and its percent decreased and PS latency increased (Fig. 2, Table 3). The diminution of PS was related to the decrease of the number of PS episodes and of their mean duration (Table 3).

TABLE 3

EFFECTS OF RO 41-9067 AND CLONIDINE ON SLEEP PARAMETERS DURING THE FIRST 4 HOURS OF THE LIGHT PERIOD (0800-1200 h)

Variables	NaCl $n = 8$	RO 41-9067 0.03 mg/kg $n = 8$	Clonidine 0.005 mg/kg $n = 8$	Clonidine ± RO 41-9067 $n = 8$
Total recording (min)	240	240	240	240
Total sleep (min)	152.4 ± 21.7	164.5 ± 15.5	116.9 ± 13.1$^{\Delta\Delta\Delta}$	130.9 ± 19.7^{***+}
W (min)	87.6 ± 21.7	75.5 ± 15.5	123.1 ± 13.1$^{\Delta\Delta\Delta}$	109.1 ± 19.7^{***+}
SWS (min)	128.8 ± 17.8	144.9 ± 13.1	116 ± 13.1	127.8 ± 19^{+}
PS (min)	23.6 ± 4.5	19.6 ± 3.3	0.9 ± 0.8$^{\Delta\Delta\Delta}$	3.1 ± 2.5^{***+}
W%	36.5 ± 9.1	31.4 ± 6.5	51.3 ± 5.4$^{\Delta\Delta\Delta}$	45.5 ± 8.2^{***+}
SWS%	84.5 ± 1.5	88.1 ± 1.4$^{\Delta\Delta\Delta}$	99.2 ± 0.7$^{\Delta\Delta\Delta}$	97.7 ± 1.8^{***+}
PS%	15.5 ± 1.5	11.9 ± 1.4$^{\Delta\Delta\Delta}$	0.8 ± 0.7$^{\Delta\Delta\Delta}$	2.3 ± 1.8^{***+}
Sleep latency (min)	30 ± 15.3	17.1 ± 5.4$^{\Delta}$	11.2 ± 10.2$^{\Delta}$	7 ± 4.2*
PS latency (min)	12.5 ± 7	23.6 ± 5.3$^{\Delta}$	103.4 ± 28.8$^{\Delta}$	82 ± 33.1*
Number of cycles and PS episodes	11.9 ± 2.6	9.6 ± 2.6	1.4 ± 1.2$^{\Delta\Delta\Delta}$	4.1 ± 2.8^{*++}
Mean duration of cycles (min)	10.5 ± 1.8	14.2 ± 3.3$^{\Delta}$	2.3 ± 5.2$^{\Delta\Delta\Delta}$	5.7 ± 3.8***
Mean duration of PS episodes (min)	2.1 ± 0.3	2.1 ± 0.3	0.2 ± 0.3$^{\Delta\Delta\Delta}$	0.6 ± 0.5***
Number of stage changes	116.9 ± 22	106.4 ± 30.5	146.9 ± 38	158.8 ± 30.6***
Number of awakenings	52 ± 10.2	47.9 ± 15.1	73.4 ± 19.1$^{\Delta}$	77.8 ± 15.7***

Drugs were given by IP route. The statistical evaluations were done with respect to NaCl (△) for RO 41-9067 alone and clonidine alone, to RO 41-9067 alone (*) and clonidine alone (+) for the combined treatment. Mean ± SD.

△,*,+$p < 0.05$; △△,**,++$p < 0.01$; △△△,***,+++$p < 0.005$.

Finally, after the combined treatment, the number of awakenings and the number of stage changes increased in comparison with RO 41-9067 alone (Table 3).

Effects of the combination of RO 41-9067 and clonidine compared with those of clonidine alone. Significant results were only observed during the first 4 h of sleep recordings. During this period, W decreased and total sleep time increased (Table 3). SWS increased while SWS% decreased in comparison with clonidine alone. After the combined treatment, PS and PS% increased (Fig. 2), reflecting an augmentation of the number of PS episodes without modification in their mean duration in comparison with clonidine alone (Table 3).

Relative to the augmentation of total sleep time, the number of sleep cycles increased (Table 3).

TABLE 4

EFFECTS OF RO 41-9067 AND CLONIDINE ON SLEEP PARAMETERS DURING THE 12 HOURS OF THE DARK PERIOD (2000-0800 h)

Variables	NaCl $n = 8$	RO 41-9067 0.03 mg/kg $n = 8$	Clonidine 0.005 mg/kg $n = 8$	Clonidine ± RO 41-9067 $n = 8$
Total recording (min)	720	720	720	720
Total sleep (min)	218,45 ± 37,91	207,3 ± 44,79	283,58 ± 36,37$^{\Delta}$	282,3 ± 34,89***
W (min)	501,55 ± 37,91	512,7 ± 44,79	436,42 ± 36,37$^{\Delta}$	437,7 ± 34,89***
SWS (min)	195,2 ± 25,33	184,55 ± 37,54	247,91 ± 33,75$^{\Delta}$	248,09 ± 29,71***
PS (min)	23,25 ± 13,94	22,75 ± 8,96	35,67 ± 9,98$^{\Delta}$	34,21 ± 10,31*
W%	69,66 ± 5,27	71,21 ± 6,22	60,61 ± 5,05$^{\Delta}$	60,79 ± 4,85***
SWS%	89,99 ± 4,82	89,35 ± 2,92	87,4 ± 3,3	87,96 ± 3,06
PS%	10,01 ± 4,82	10,65 ± 2,92	12,6 ± 3,3	12,04 ± 3,07
Sleep latency (min)	27,79 ± 23,31	37,54 ± 33,95	13,92 ± 22,08	8,58 ± 7,42*
PS latency (min)	9,75 ± 4,15	11,13 ± 9,02	7,67 ± 4,67	10 ± 7,48
Number of cycles and PS episodes	15,5 ± 10,31	13,88 ± 6,11	22,75 ± 5,99	22,25 ± 5,52*
Mean duration of cycles (min)	8,02 ± 4,05	9,24 ± 2,01	7,94 ± 1,11	7,87 ± 1,32
Mean duration of PS episodes (min)	1,45 ± 0,65	1,79 ± 0,38	1,59 ± 0,19	1,58 ± 0,31
Number of stage changes	264,38 ± 57,62	262 ± 36,24	390,5 ± 60,57$^{\Delta\Delta\Delta}$	378,25 ± 66,97***
Number of awakenings	123,25 ± 26,11	123,63 ± 15,86	183,75 ± 30,4$^{\Delta\Delta}$	177,88 ± 32,65**

Drugs were given by IP route. The statistical evaluations were done with respect to NaCl (△) for RO 41-9067 alone and clonidine alone, to RO 41-9067 alone (*) and clonidine alone (+) for the combined treatment. Mean ± SD.

△,*,+$p < 0.05$; △△,**,++$p < 0.01$; △△△,***,+++$p < 0.005$.

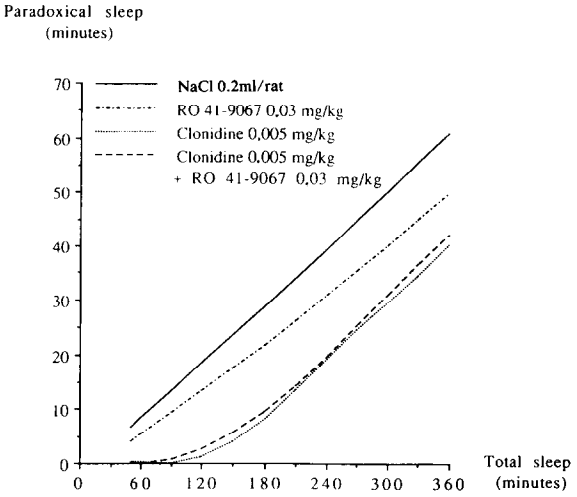

FIG. 2. General trend of paradoxical sleep during the light period under the effect of clonidine IP and RO 41-9067 IP applied alone or successively.

Effects of the Combination of RO 41-9067 and Clonidine During the Dark Period

Because of the short lasting effects of RO 41-9067, the effects of the combination of RO 41-9067 and clonidine were similar to those of clonidine alone (Table 4).

DISCUSSION

The results of this study are in harmony with those of microdialysis in cat's striatum demonstrating a dose-dependent decrease in dopamine release after RO 41-9067 (24). In our experiment, after RO 41-9067, PS also decreased dose dependently, suggesting a possible role for dopamine in the production of this parameter.

A mixed D_1/D2 agonist apomorphine, D_2 agonist quinpirole and bromocriptine (12,18,19) have been shown to produce biphasic effect in rats, increasing at low doses and decreasing PS at high doses. Low doses of the agonist that stimulate D_2 presynaptic dopamine receptors, induce a negative feedback, and, as a consequence, the synthesis and liberation of dopamine decrease (16) while high doses of D_2 agonist stimulate postsynaptic DA receptors. However, this biphasic effect did not seem to occur in rats after administration of RO 41-9067.

RO 41-9067 seems to have a similar effect on PS than that observed with the pergolide, another D_2 presynaptic receptors agonist (18). During the light period, they both dose dependently decreases PS and at high doses increases waking.

Another major effect of RO 41-9067 seems to be the stabilization of sleep during the light period, which was reflected by a decrease of number of awakenings. Moreover, during the dark period, the high dose of RO 41-9067 showed an hypnogenic action. The sedative effect of RO 41-9067 is well correlated with significant reductions in locomotor activity in rats (21) and corresponds well with a D_2 presynaptic action, reduction of synthesis and liberation of DA.

In the prefrontal cortex, the concentration of DA is low and it is synthetized faster than in the subcortical area where dopamine exists in higher amount (25). This could mean that the rate of inhibition of dopamine release is probably different between cortical and subcortical regions. The type, the density, or sensitiveness of receptors could also underlye the variation in the inhibition of dopamine release.

The highest dose of RO 41-9067 also caused significant augmentations in waking and decrease in PS. Because RO 41-9067 also binds to 5-HT_{1A} receptors, the possibility should be considered that the compound, in addition to D_2 receptors, may also interfer with a lower affinity with 5-HT_{1A} receptors (21).

It has also been reported that, at low doses, clonidine produces a negative feedback on noradrenergic neurons that induces an inhibition of noradrenaline release entailing a decrease of noradrenaline synthesis (23). This central noradrenergic depletion could be too high to allow PS setting. In parallel, low doses of clonidine may decrease noradrenaline release in the locus coeruleus and PS, suggesting an involvement of the locus coeruleus in the realization of PS.

The combination of RO-clonidine prevent the decrease of total sleep time as well as the decrease of PS induced by clonidine alone. During the light period, the significant attenuation of the effects of clonidine alone on different sleep parameters after the combination of RO-clonidine, strongly suggests an interaction between noradrenergic and dopaminergic activities. This observation is in agreement with different data showing the existence of a dopaminergic pathway from the striatum to the pars reticulata and to the locus coeruleus (9,17,26).

Considering that RO 41-9067 has a certain affinity for α_2 adrenoceptors (21), the results of this study is in favour of an interaction between dopaminergic and noradrenergic systems in the regulation of sleep.

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