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Effects of Clonidine and α-Methyl-*p*-Tyrosine on the Carbachol Stimulation of Paradoxical Sleep

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MASTRANGELO, D., Z. DE SAINT HILAIRE-KAFI AND J.-M. GAILLARD. *Effects of clonidine and α-methyl-p-tyrosine on the carbachol stimulation of paradoxical sleep.* PHARMACOL BIOCHEM BEHAV 48(1) 93–100, 1994.– Acetylcholine promotes paradoxical sleep (PS), but the role of noradrenaline in this stimulation is controversial. The relationship between cholinergic and noradrenergic systems in the production of PS was investigated in the rat implanted on a continuous basis for sleep recordings. Stimulation of PS was obtained with microinjections of carbachol (1 μg) into the pontine reticular formation. In the presence of the alpha$_2$-agonist clonidine (5 μg/kg, IP), the carbachol activation of PS was abolished. This stimulation also disappeared when the animals were pretreated with alpha-methyl-paratyrosine (150 mg/kg, IP), an inhibitor of catecholamine synthesis. Thus, carbachol stimulation appeared inefficient when brain noradrenergic activation was decreased. This observation supports the view that the realization of PS by the cholinergic system requires a certain level of noradrenergic activity.

Cholinergic system Noradrenergic system Carbachol Clonidine Alpha-methyl-para-tyrosine
Paradoxical sleep Rat

IT is generally accepted that cholinergic mechanisms are involved in the initiation and the maintenance of paradoxical sleep (PS) [for reviews, see (3,13,20)]. However, if administration of cholinergic compounds into the pontine tegmentum promotes PS, it is not yet established whether this effect is direct or necessitates the presence of another transmitter such as noradrenaline (NA). Contradictory data concerning the catecholaminergic systems do not give a clear picture of their implication in PS. Chemical and electrolytic lesion studies, although not always concordant, led to the conclusion that the noradrenergic system is not necessary for the induction of PS [for reviews, see (12,20,33,40)]. Cooling of the locus coeruleus, inducing temporary inactivation without destruction of neuronal elements, clearly enhances PS (8); according to this result, the noradrenergic system plays, in fact, a permissive influence: its activity prevents the realization of PS, which appears only when this activity stops. Electrophysiological studies indicate that the locus coeruleus neurons decrease or cease firing during PS, confirming a preventive instead of a facilitory or generative effect upon PS, as described by the reciprocal interaction model of PS generation (2,17,18,41).

The large number of pharmacological studies, aimed at modifying noradrenergic activity (including administration of NA precursors, NA synthesis inhibitors, NA receptors agonists and antagonists, NA release stimulants, NA uptake inhibitors, monoamine oxydase inhibitors, catechol-O-methyl transferase inhibitors), give a seemingly confusing set of data [for reviews, see (1,9,12,31–33,45)]. Nevertheless, a fair number of these results suggests that some degree of noradrenergic activity is required for the appearance of PS. It is not yet determined which kind of noradrenergic neurons could be implicated in this activity, and the question of the interrelationship between ACh and NA in PS induction is not fully elucidated. Histological evidence shows that cholinergic and monaminergic cells are intermingled in several ponto-medullary neuron groups, and indicates the possibility of reciprocal connections between these two neuronal systems (20, 41,50).

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The α_2-adrenergic agonist clonidine has been used to interfere with noradrenergic activity. In experiments with pontine brain slice preparations, it has been shown that clonidine hyperpolarizes the exposed neurons, a result compatible with the reciprocal interaction model (15). It is conceivable that a postsynaptic α_2-receptor mechanism could inhibit PS, although brain slice preparations do not permit determination of the role of the neurons studied in PS generation. It is well established that clonidine decreases or stops the firing of noradrenergic cells and inhibits the release of the neurotransmitter from the terminals, presumably via an autoreceptor mechanism (7,46). Both in man and animals, systemic injection of clonidine has been shown to inhibit PS [for references, see (12,21,27)], which appears to conflict with the reciprocal interaction model.

In an attempt to clarify the role of noradrenergic neurons in PS, we have studied the effect of systemic injection of clonidine and alpha-methylparatyrosine (αMPT), specific inhibitor of tyrosine hydroxylase (44), on the facilitation of PS induced by intracerebral injection of carbachol in free-moving, unanaesthetized rats. Some preliminary results of these experiments have been published (28).

METHOD

Male Wistar rats, weighing 270–350 g, were surgically implanted under pentobarbital anaesthesia (55 mg/kg) with three electrodes for electroencephalogram (EEG) and two in the dorsal neck muscles for electromyogram (EMG). The EEG electrodes were made with chloridized silver wire terminating in a 1 mm diameter sphere which was pushed into a hole made in the skull. These electrodes were positioned laterally over the frontal cortices (2 mm lateral): one electrode 2 mm anterior and another 3 mm posterior to the bregma, with a ground electrode between them.

In addition, each rat received unilaterally a 0.6 stainless steel guide through a hole drilled into the calvarium. Using the Paxinos and Watson atlas (37) as reference, the guide was targeted to the pontine reticular formation, so that the tip of the cannulae inserted through it lies between the nuclei pontis oralis and caudalis, near the trigeminal motor nucleus: 0.2 mm anterior to the interaural line, 1.4 mm lateral to the midline, and 8.5 mm dorsoventral from the surface of the skull. The incisor bar of the stereotaxic instrument (David Kopf Instrument, Tujunga, CA) was set at 5.5 mm below the interaural zero. The electrodes, bound to a connector, and the guide were then fixed onto the skull with the aid of stainless steel screws and dental cement. A removable rod of equal length was placed into the guide to prevent occlusion.

After surgery, the rats were housed individually in a room with a daylight period maintained between 0800 and 2000 h. A minimum of 10 days later, the animals were placed in individual cages with free access to food and water, and connected to an EEG polygraph (Grass, Quincy, MA) for two sessions of 5 days separated by 1 week. Each session consisted of 2 adaptative days, followed by 2 test days, separated by an intervening day without any treatment. On the test days, the recordings began at 0800 h and lasted for 11 h. Three stages of EEG activity—waking (W), slow wave sleep (SWS), and paradoxical sleep (PS)—were visually scored according to conventional criteria in 20 s epochs, allowing the calculation of parameters such as total duration, latency, and percentage of each stage. All scoring was done blind to drug condition. To study the temporal organization of sleep during the experiments, the cumulated occurrences of each stage were counted in 10 min epochs; the general trends were then calculated as already shown by a curvilinear regression using orthogonal polynomials (11). The evolution of W was given with respect to total recording time, and the evolution of SWS and PS with respect to total sleep (total sleep = recording time after deduction of W). In this way, the temporal organization of the states of vigilance was described by a curve characterized by three parameters: the level (average, degree 0 of the polynomial), the slope (linear regression, degree 1), and the curvature (parabolic regression, degree 2). These three parameters were calculated for each vigilance state in each recording and used for statistical estimation (11). Statistical significance of the data was assessed by means of the two-tailed Student's t-test.

Intracerebral injection was delivered using a 0.3 mm stainless steel cannula which passed 2 mm beyond the tip of the implanted guide. This injection cannula was connected to a 5 μl Exmire syringe (Ito Corp., Fuji, Japan) by 30 cm of flexible plastic tubing. All injections were 0.5 μl in volume delivered over 3 min, and the injection cannula was left in place for an additional 2 min before being withdrawn. The results of the various treatments were compared with those obtained with similar administration of NaCl 0.9%, with the view to preventing carry-over effects of the treatments.

The following types of injection were performed on sixteen animals: a) saline solution (NaCl 0.9%) administered successively intraperitoneally (IP, 1 ml/kg) and via the cannula (IC, 0.5 μl) as controls; b) saline solution IP (1 ml/kg), and carbamylcholine IC (Carbachol, Sigma, Buchs, Switzerland, 1 μg in a 0.5 μl volume of saline); c) clonidine IP (5 μg/kg, Sigma) dissolved in saline (1 ml/kg), and saline IC (0.5 μl); d) clonidine IP (5 μg/kg) dissolved in saline (1 ml/kg) and carbamylcholine IC (1 μg/0.5 μl). Eight of these rats received two more treatments: e) alpha-methyl-para-tyrosine IP (αMPT, Hoffmann-La Roche, Basel, Switzerland, 150 mg/kg in 1.5 ml/kg) and saline IC (0.5 μl); f) αMPT IP (150 mg/kg) and carbamylcholine IC (1 μg/0.5 μl). The IP injections were effected at 0830 h, with the IC injections following about 30 min later. Hypothermia induced by clonidine was prevented by keeping the animals at 25°C.

At the end of the experiment, each rat was sacrificed and 20 μl of a solution of Evan's blue (5 mg/ml) was injected in the cannula. The formalin-fixed brain was sectioned sagittally at 50 μm, and the injection site was verified on the mounted sections.

RESULTS

The histological verification showed that the cannula lay, as expected, between the nuclei pontis oralis and caudalis in each of 10 rats among the 16 used. Otherwise, the cannula was 1 mm anterior to the nucleus oralis in five rats, and 2 mm posterior to the nucleus caudalis in one rat. Four animals did not respond to the injections of carbachol: one with the cannula anterior to the designed site and three with the cannula correctly implanted (probably because of clots blocking the guides). Injections of carbachol into the rat with the caudally implanted cannula produced unusual rotation of the animal. The four rats showing no response to carbachol as well as the animal showing an abnormal behavioral response were not included in the analysis of the carbachol and the combined treatment.

Table 1 gives the effects of carbachol on the sleep parameters in the remaining 11 rats. The injection of 1 μg produced a marked increase of PS and decreased SWS, and slightly increased the total time of waking and the number of awaken-

TABLE 1

MODIFICATION OF SLEEP PARAMETERS INDUCED BY CARBACHOL AND CLONIDINE, APPLIED ALONE OR SUCCESSIVELY

Parameters	Controls $n = 16$	Carbachol 1 μg/0.5 μl $n = 11$	Clonidine 5 μg/kg $n = 16$	Clonidine + Carbachol $n = 11$
Total sleep (min)	350 ± 29	321 ± 35*	315 ± 49*	318 ± 35
Waking (min)	158 ± 29	187 ± 35*	193 ± 49*	190 ± 35
Slow wave sleep (SWS, min)	292 ± 30	247 ± 34†	277 ± 37	280 ± 27*
Paradoxical sleep (PS,min)	58 ± 6	74 ± 8†	38 ± 15†	38 ± 16†
Sleep latency (min)	30 ± 18	49 ± 22	40 ± 52	46 ± 34
PS latency (min)	17 ± 10	11 ± 8*	88 ± 59†	85 ± 64†
Waking (%)	31 ± 6	37 ± 7*	38 ± 10*	37 ± 7
SWS (%)	83 ± 2	77 ± 3†	89 ± 4†	88 ± 5†
PS (%)	17 ± 2	23 ± 3†	12 ± 4†	12 ± 5†
Number of sleep cycles and PS episodes	29 ± 5	38 ± 5†	23 ± 10	24 ± 11†
Mean duration of sleep cycles (min)	10.5 ± 1.7	7.7 ± 1.6†	8.9 ± 1.3‡	8.9 ± 2.0
Mean duration of PS episodes (min)	2.1 ± 0.3	2.1 ± 0.3	1.7 ± 0.2†	1.6 ± 0.4†
Number of awakenings	125 ± 26	146 ± 37	181 ± 41†	156 ± 22

The statistical estimation was done with respect to the controls for carbachol and clonidine alone, respectively, and to carbachol alone for the combined treatment.

Mean ± SD.

*$p < 0.05$.

†$p < 0.005$.

‡$p < 0.01$.

ings. During 20 to 80 min following the injection, carbachol induced a period of continuous quiet waking; this resulted in an average increase in the sleep latency by about 20 min. After the first appearance of SWS, however, the PS latency was shorter than in the controls. The level and the slope of the general trend of PS obtained after application of carbachol were significantly higher than in control recordings (Table 3A); in addition, the effect of the cholinergic agonist was sustained during the entire duration of the recording (Fig. 1). This increase was due to a rise in the number, but not in the duration of the PS episodes (Table 1). At the same time, the number of sleep cycles was increased and their mean duration shortened.

The administration of a dose of 5 μg/kg of clonidine resulted essentially in a significant decrease in PS (Table 1), whereas the other stages were much less affected. During the 2-6 h following the injection, the dominating EEG pattern was somewhat different from the drowsy state of control rats: clonidine-induced bursts of large amplitude waves (400-600 μV; mean frequency: 6 Hz; burst duration: 1-5 s). During these periods with bursts apparition, the rat kept generally still but remained reactive to external stimuli. These epochs were then included in the category of wakefulness. Thus, after application of clonidine, the total time of waking and the number of awakenings increased, and, therefore, the total sleep decreased. Because of the loss of PS, SWS, yet not affected, was proportionally more important. In addition, clonidine lengthened the latency of PS by nearly 60 min, and reduced the duration of the episodes. The general trend of PS showed also that clonidine induced an initial suppression of PS, followed by a reappearance at a higher production rate (Fig. 1). This rate is described by the slope of the general trend which, 4 h after the injection, became steeper under clonidine than in the controls. Nevertheless, this higher production rate did not compensate for the initial loss, and the total amount of PS was finally lower than that in control conditions (Fig. 1 and Table 3A).

The results obtained with the successive application of clonidine and carbachol did not differ from clonidine alone (Table 1). The increase of PS produced by carbachol disap-

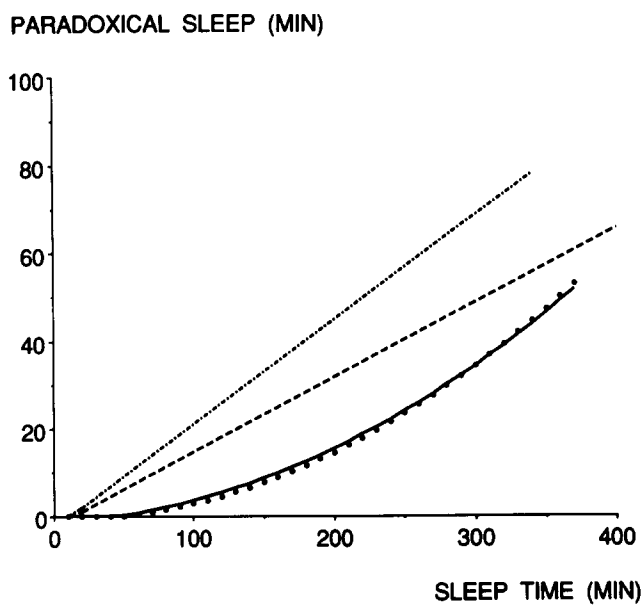

FIG. 1. General trend of paradoxical sleep under the effect of carbachol and clonidine applied alone or successively. --- controls; ·-·-·-· carbachol 1 μg/0.5 μl IC; · · · clonidine 5 μg/kg IP; — clonidine + carbachol. Abscissa: total sleep time (min, waking discarded).

peared when the rats were pretreated with clonidine, and all sleep parameters were similar to those obtained with clonidine alone (no statistical difference between these two groups). Moreover, the general trend of PS obtained with the carbachol-clonidine combination and with clonidine alone were clearly superimposable (Fig. 1): no difference in level, slope or curvature was perceptible between them (Table 3A).

The effects of αMPT are given on Table 2. A single injection of 150 mg/kg did not induce any significant alteration of sleep, except for an increase in PS latency. Pretreatment with αMPT not only suppressed the facilitation of PS normally seen after carbachol, but induced an important reduction of this stage. As already mentioned, the facilitation of PS under carbachol resulted in an increase in the number of PS episodes. When carbachol was injected after αMPT, this number was lowered below the control value. Moreover, the duration of the PS episodes, which was affected neither by carbachol nor by αMPT alone, was also reduced by the combination of these two agents.

The action of carbachol, alone or after pretreatment of αMPT, on the evolution of PS is shown in Fig. 2 and Table 3B. At the dose used, αMPT had no significant effect by itself. However, when carbachol was injected after αMPT, the increase of the general trend observed with carbachol alone was replaced by a long-lasting depression in the production of PS.

After pretreatment with αMPT, carbachol slightly increased the percent of SWS, while it decreased it when applied alone (Table 2). W was unaffected or only slightly affected by αMPT and carbachol alone, but the successive application of these two compounds seemed to increase the number of awakenings and total waking (Table 2).

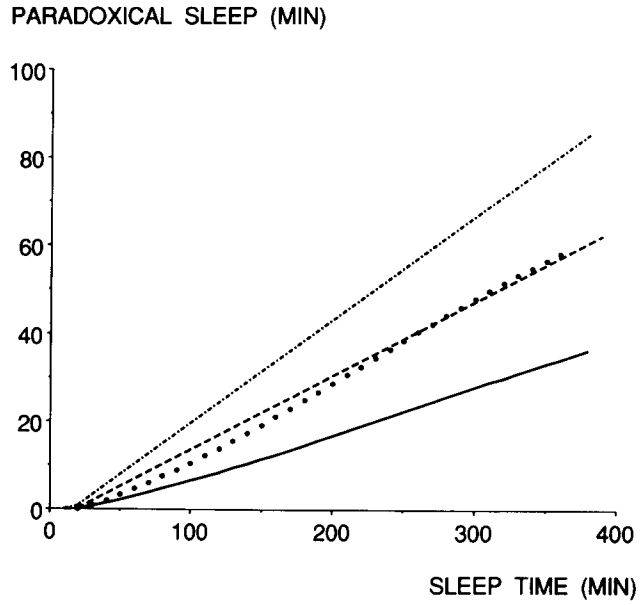

FIG. 2. Effect of pretreatment with 150 mg/kg of α-methylparatyrosine (αMPT) 30 min before carbachol on the general trend of paradoxical sleep. --- controls; ·-·-·- carbachol 1 μg/0.5 μl IC; · · · αMPT IP; — αMPT + carbachol. Abscissa: total sleep time (min, waking discarded).

DISCUSSION

The purpose of this study was to determine the possibility of noradrenergic participation in the expression of the cholinergic activation of PS. One important result was that the effects of carbachol microinjections into the pontine reticular formation of the rat were totally abolished in the presence of the α_2-agonist clonidine on one hand, and of the catecholamine synthesis inhibitor αMPT on the other hand. Thus, when brain noradrenergic activity was decreased, cholinergic stimu-

TABLE 2

MODIFICATION OF THE EFFECT OF CARBACHOL
30 MINUTES AFTER PRETREATMENT WITH αMPT 150 mg/kg

Parameters	Controls $n = 8$	Carbachol 1 μg/0.5 μl $n = 6$	αMPT 150 mg/kg $n = 8$	αMPT + Carbachol $n = 6$
Total sleep (min)	381 ± 16	346 ± 35	365 ± 35	317 ± 75
Waking (min)	160 ± 16	195 ± 35	176 ± 35	224 ± 75
Slow wave sleep (SWS, min)	319 ± 19	268 ± 31*	303 ± 31	286 ± 61
Paradoxical sleep (PS, min)	62 ± 7	78 ± 8*	61 ± 19	31 ± 17*
Sleep latency (min)	40 ± 17	58 ± 21	41 ± 8	53 ± 20
PS latency (min)	17 ± 9	14 ± 9	31 ± 7*	39 ± 20†
Waking (%)	30 ± 3	36 ± 7	33 ± 6	42 ± 14
SWS (%)	84 ± 2	77 ± 2*	83 ± 5	91 ± 3*
PS (%)	16 ± 2	23 ± 2*	17 ± 5	9 ± 3*
Number of sleep cycles and PS episodes	32 ± 6	41 ± 5†	34 ± 9	22 ± 10*
Mean duration of sleep cycles (min)	10.8 ± 2.1	7.8 ± 1.8†	8.9 ± 1.6	9.1 ± 3.3
Mean duration of PS episodes (min)	2.1 ± 0.3	2.1 ± 0.4	1.9 ± 0.3	1.4 ± 0.2*
Number of awakenings	121 ± 17	144 ± 35	127 ± 25	172 ± 37

The statistical estimation was done with respect to the controls for carbachol and αMPT alone, respectively, and to carbachol alone for the combined treatment.

Mean ± SD.

*$p < 0.005$.

†$p < 0.05$.

TABLE 3

COEFFICIENTS OF THE GENERAL TREND OF PARADOXICAL SLEEP OBTAINED WITH CARBACHOL AND CLONIDINE APPLIED ALONE AND SUCCESSIVELY (A), OR WITH CARBACHOL AND αMPT APPLIED ALONE OR SUCCESSIVELY (B)

	Treatment	Dosage	*n*	Level	Slope	Curvature
A	Saline	Equiv.	16	34.0 ± 1.3	1.76 ± 0.08	0.003 ± 0.003
	Carbachol	1 μg/0.5 μl	11	42.6 ± 1.8*	2.51 ± 0.14*	0.003 ± 0.003
	Clonidine	5 μg/kg	16	20.9 ± 1.9*	1.78 ± 0.07	0.032 ± 0.008*
	Clonidine + Carbachol	5 μg/kg 1 μg/0.5 μl	11	22.2 ± 2.3*	1.79 ± 0.09*	0.031 ± 0.009*
B	Saline	Equiv.	8	30.0 ± 2.3	1.68 ± 0.30	0.008 ± 0.008
	Carbachol	1 μg/0.5 μl	6	38.1 ± 4.6*	2.35 ± 0.21*	0.007 ± 0.007
	αMPT	150 mg/kg	8	28.8 ± 8.7	1.84 ± 0.54	0.008 ± 0.006
	αMPT + Carbachol	150 mg/kg 1 μg/0.5 μl	6	15.0 ± 7.7*	0.89 ± 0.57*	−0.008 ± 0.002

Numbers without dimension. The statistical estimation was done with respect to the controls for carbachol, clonidine and αMPT alone, respectively, and to carbachol alone for the combined treatments.
Mean ± SD.
*$p < 0.005$.

lation appeared unable not only to affect PS, but also to reverse the action of clonidine. The possibility of postsynaptic α_2-adrenoceptor stimulation by clonidine should be considered. However, the abolition of the carbachol response after both clonidine and αMPT is not in favor of this possibility. These observations support the idea that the effect of carbachol on PS depends on a certain level of noradrenergic activity.

Microinjections of carbachol into the pontine reticular formation of the rat, between the nuclei pontis oralis and caudalis, resulted as expected in a shortening of PS latency and in an increase in the production of this stage. The stimulating effect was sustained during the 11 h of recording, probably due to the slow catabolism of this cholinergic agonist. In the rat, pontine reticular formation appears to be one of the most effective sites for promoting PS with carbachol infusions (14). The present results are also compatible with those obtained in other species, where microinfusions of cholinergic compounds into the pontine reticular formation support this pharmacological model of PS [for reviews, see (3,20)].

Administration of the selective α_2-adrenoceptor agonist clonidine induced a biphasic effect, as already described (21): clonidine first inhibited PS and this initial inhibition was followed by a rebound in the last few hours of the recording. This type of response was attributed to the half-life of clonidine of approximately 90 min in the rat when injected IV (19). At the dose used, it is known that clonidine blocks the firing activity of the noradrenergic system (probably by stimulating somatodendritic α_2-autoreceptors located on noradrenergic cell bodies), and also inhibits the release of NA from terminals (through the activation of presynaptic α_2-autoreceptors); consequently, the activity of these neurons decreases (7,10,46). The effect of clonidine on PS seems to be realized in the dorsal pontine tegmentum, because in the cat local microinjections suppress PS in the same way as systemic injections (48). In this case, the action of clonidine is observed only when the site of injection is restricted to the perilocus coeruleus α, a region considered as one of the optimal sites for PS induction with carbachol administration (50), and known to contain α_2-adrenoceptor binding sites (53).

The PS decrease observed after application of clonidine appears to be the result of a direct action on PS, because the other stages were practically not modified. A possible contribution of the hypotensive property of clonidine seems unlikely, because several studies have shown that alterations in the sleep–wake cycle were not dependent on changes in cardiovascular activity (38,39,43). Clonidine is known to induce hypothermia when injected systemically (25), and hypothermia affects PS (36). However, in our study the animals were kept at 25°C to limit this effect. Moreover, the suppression of PS by microinjection of clonidine in the dorsal pontine tegmentum of the cat is not associated with an alteration of brain temperature (48).

The possibility that clonidine acts on α_2-adrenoceptors present on nonnoradrenergic neurons should be considered. Clonidine has very little effect on serotonin and dopamine, and these effects appear to be brought about via noradrenergic neurons (23,46,52). Brain histamine H_2-receptors can also be stimulated by clonidine; several data, however, do not support a role for these receptors in the clonidine-induced EEG and the behavioral changes [see (30) for review]. Thus, compared to NA, it would seem that other biogenic amines do not play an important part in the PS modifications observed after application of low doses of clonidine.

Associations between noradrenergic, cholinergic, and GABAergic cells are found in the mediodorsal pontine tegmentum and, besides direct interactions between noradrenergic and cholinergic neurons, indirect interactions may occur via the intermediary of local GABAergic neurons (20). It cannot be excluded that clonidine may affect these indirect interactions, because it was shown that clonidine increases the release of GABA from the guinea pig brain surface, and that GABA modulates the cortical release of ACh (34,47).

Finally, the possibility that clonidine affects PS through anticholinergic side effects is suggested by its known ability to produce a marked inhibition of ACh synthesis and release through prejunctional α_2-adrenoceptors at peripheral and central nerve terminals (5,42,49,51). However, such effects are obtained in the rat brain with doses of clonidine much higher [30 μg/kg IV, or 100–2000 μg/kg IP; (26)] than those affecting PS (5 μg/kg IP in the present study).

Most importantly, a decrease in ACh synthesis or release

induced by clonidine should be compensated by the application of the cholinergic agonist carbachol. However, the present investigations have shown that pontine microinjections of carbachol are unable to modify the effect of clonidine, which suggests that clonidine does not affect—directly or indirectly—the cholinergic system implied in the PS stimulation. The present results are supported by Tononi et al. (48), who have shown that clonidine injected directly into the cat dorsal pontine tegmentum (0.025 μg in 0.25 μl saline bilaterally) also prevent the effect of carbachol. Because clonidine suppresses PS after systemic or local intracerebral injections, it seems unlikely that an experiment with intracerebral application of both clonidine and carbachol would give essentially different results. However, this experiment could be done.

It should also be noted that at least three α_2-adrenoceptor subtypes have been recognized (6), which can be functionally divided into prejunctional, postjunctional receptors, and somato-dendritic autoreceptors. The precise role played by these distinct receptors in the PS control is actually not explained, but the existence of multiple α_2-adrenoceptors could partially explain the seemingly unclear set of data obtained with nonspecific pharmacological compounds, as the lack of antagonism between clonidine and α-antagonists on PS [see (12) or (27)].

Furthermore, the clonidine-induced PS decrease could also be the result of an effect on sites other than α_2-adrenoceptors that may interfere with PS. Effectively, clonidine can also bind to what are usually called imidazoline binding sites present in the brain stem (4). Thus, it would be interesting to test the effect on PS of α_2-agonists devoid of imidazoline-like structure, such as xylazine, BHT-920, or BHT-933. These compounds have been shown to increase canine cataplexy, whereas clonidine (up to 4 μg/kg IV) does not (35). However, α-adrenergic antagonists such as phentolamine or yohimbine prevent the PS decrease induced by clonidine injected into the dorsal pontine tegmentum (48), which suggests that the PS clonidine action is mediated by adrenoceptors rather than imidazoline binding sites.

The present results with clonidine are supported by those obtained with αMPT. It has been established for several years that αMPT is a reversible and competitive inhibitor of tyrosine hydroxylase, the rate-limiting step in the synthesis of the catecholamine (CA) family (44). Because αMPT affects both dopamine and NA synthesis, experiments with this inhibitor do not allow differentiation between the roles of these two amines. In the rat, the administration of a single dose of 100 mg/kg causes a 45% decrease in the level of NA and a 74% decrease in the level of dopamine, while the respective levels of serotonin and ACh do not change (24). Low doses of α-methyl-tyrosine are effective for more than 8 h in reducing noradrenaline synthesis, without toxicity (44). Administration of αMPT to different species has given conflicting results concerning its effect on PS [see (12) for review]. Such discrepancies disappear if the dose–response relationship of αMPT is examined: in the rat, a low dose of this compound tends to enhance the production of PS, while a higher dose decreases it (22,29). These data suggest that a marked decrease of endogenous CA interferes with the production of PS.

Following previous experiments in this laboratory (22), a single dose of 150 mg/kg of αMPT has been used in the present study. At this dose, αMPT produces only a partial decrease of endogenous CA, as revealed by histofluorescence techniques showing that the depletion of brain CA is not complete after such treatment (22). In the present experiment, 150 mg/kg of αMPT was almost ineffective by itself on sleep characteristics. On the other hand, if the microinjections of carbachol were preceded by pretreatment with αMPT, not only did the PS facilitation no longer appear, but it was replaced by a marked decrease. It is actually not known why this compound not only prevented but also reversed the carbachol effect. A possible explanation is that inhibition of CA synthesis with the dose of αMPT used in this study leaves enough endogenous CA to ensure undisturbed NA activity in basal conditions. However, when higher demand in NA systems is exerted by cholinergic stimulation, they cannot sustain it due to the limited availability of NA under conditions of synthesis inhibition.

Taken together, the results of the present study suggest that the effect of cholinergic stimulation by carbachol on PS necessitate the participation of brain CA systems. The results with clonidine indicate that this CA is probably NA. Thus, a certain noradrenergic activity would be essential for the expression of cholinergic activation. Pharmacological evidence was used to suggest that a controlled or restricted enhancement of the central noradrenergic activity promotes the emergence of PS, whereas a more pronounced enhancement would support waking. The possibility that cells of the locus coeruleus are mostly related to wakefulness and that other cells of the subcoeruleus area or other nuclei may remain active during PS has already been proposed (12,16). It should be emphasized that this possibility is not incompatible with the reciprocal interaction model, which however might have to be supplemented by taking into consideration additional factors, to account for pharmacological data obtained with agents that interfere with α_2-adrenoceptors.

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