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Modulation of desynchronized sleep through microinjection of α_1-adrenergic agonists and antagonists in the dorsal pontine tegmentum of the cat

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Abstract. Noradrenaline is involved in the regulation of the sleep/waking cycle by acting through various receptor types. In previous studies we investigated the role of β- and α_2-adrenergic receptors through local microinjections of various drugs into the dorsal pontine tegmentum (DPT) of the cat. This region is known to be crucially involved in desynchronized sleep execution. In this study we examined the role of α_1-adrenergic receptors. The α_1-agonist methoxamine and the α_1-antagonist prazosin were injected into the DPT of freely moving, unanaesthetized cats. We found that methoxamine notably reduced desynchronized sleep, and that this effect was both dose-dependent and site-specific. These effects were prevented by the subsequent injection of prazosin. On the other hand, the injection into the DPT of prazosin alone produced scarce or inconsistent effects on the sleep/waking cycle.

Key words: REM sleep – Central α_1-adrenoceptors – Methoxamine – Prazosin – Pontine reticular formation – Locus coeruleus

Introduction

The regulation of desynchronized sleep (DS) is significantly modulated by brain noradrenergic systems. For instance, several systemic injection experiments have shown that α_1-, α_2-, and β-adrenoceptor agonists and antagonists can vary the amount of time spent in DS in several animal species. The executive and regulatory mechanisms of DS are relatively localized within the dorsal pontine tegmentum (DPT). The electrolytic, thermolytic, or chemical destruction of this region disrupts DS, and various agents, when infused locally in this area, are able to affect DS (references in [8]). According to the reciprocal-interaction model of Hobson and McCarley [7], the generation of DS depends upon the activity of two neuronal groups located in the DPT, DS-on and DS-off cells. Cholinergic and/or cholinoceptive DS-on cells are thought to be directly responsible for DS execution, while DS-off noradrenergic neurons in the locus coeruleus (LC) would play a permissive role. During waking and synchronized sleep (SS) noradrenergic DS-off cells would tonically inhibit DS-on cells, while the cessation of noradrenergic discharge during DS would allow the cholinoceptive neurons to trigger a DS episode.

Previous experiments from this laboratory have tested some of the assumptions of this model by examining the effects of the microinjection of β- and α_2-adrenoceptor agonists and antagonists into the DPT [20, 21]. Bilateral injections of the β-agonist isoproterenol reduced DS, while the β-antagonist propranolol consistently enhanced it, largely because of an increased number of DS episodes [20]. Bilateral injections of the α_2-agonist clonidine produced an almost complete suppression of DS [21]. In all cases, the results were dose-dependent and site-specific. With the present study, we aimed at exploring the contribution of α_1-adrenoceptors to DS regulation. That such receptors may also be involved in DS regulation is suggested by systemic injection experiments, in which α_1-noradrenergic agents were found to modify the sleep/waking cycle in several species including man [10, 12, 17]. The results of systemic injections are, however, scarcely conclusive, owing to peripheral and/or secondary actions of the drugs. For these reasons, we applied the chemical microinjection technique to infuse the α_1-agonist methoxamine and the α_1-antagonist prazosin directly into the DPT of freely moving, unanaesthetized cats. Preliminary results of these experiments have already been published [4].

Material and methods

Ten adult cats (3–5 kg) of either sex under pentobarbital anaesthesia (35 mg/kg, i.p.) were implanted with chronic electrodes for recording the electroencephalogram (EEG), electrooculogram and electromyogram (EMG) from dorsal neck muscles, following the indications of Ur-

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sin and Sterman [22]. Bipolar electrodes were placed in the lateral geniculate nucleus and in the dorsal hippocampus to record the ponto-geniculate-occipital (PGO) activity and the theta rhythm, respectively. Two parallel 24-gauge stainless-steel guide tubes were implanted through the forebrain in a parasagittal plane, at an angle of 30° from the vertical, passing rostrally to the tentorium and reaching the brainstem at the stereotaxic depth of H+1 and laterality of L 2.5 to 2.8. The tubes were kept pervious by removable styluses. After recovery from surgery the animals were adapted to a sound-proof and dimly illuminated recording cage until the sleep-waking parameters were in the published norms (references in [22]). In the cage, air composition and temperature were kept constant, and food and water were available ad libitum. All recording sessions took place between 2 p.m. and 9 p.m. Before the first injection, four or more baseline recording sessions were performed for each animal. Thereafter, the cats were injected with drugs or saline (controls) at intervals of about 4 days. During the injections the animals were head-restrained by hand. A 31-gauge stainless-steel cannula, connected to a 1-µl manually driven syringe (Hamilton, Bonaduz, Switzerland), was inserted through the guide tubes aimed at various sites within and around the DPT. In all instances, 0.25 µl solution was delivered over 60 s. Small volumes and slow times of injection were chosen because they help to minimize drug diffusion (cf. [20] for references). Also, to reduce back-diffusion, the cannula was left in place for an additional 60 s after the injection. Before and after each injection the cannula was checked to exclude leakage or occlusion. When the drugs were given bilaterally, the interval between right and left injections was less than 3 min. Since saline injections into this region are known not to modify sleep/waking parameters, in most control recordings the injection of saline was simulated in order to avoid additional mechanical damage to the target area (cf. [20] for details). The drugs used were: methoxamine-hydrochloride (Sigma, St. Louis, MO, USA), prazosin-hydrochloride (Sigma), and carbachol (Sigma). Methoxamine was diluted with saline to reach concentrations of 16, 8, 4, and 1 µg/µl. Prazosin solutions were prepared as follows: 10 mg prazosin was added to 0.1 ml *N, N*-dimethylacetamide, gently heated and diluted in saline to obtain concentrations of 10, 1, 0.1, 0.5, 0.01, and 0.001 µg/µl. Carbachol was diluted with saline and used at concentration of 0.4 µg/µl. On the average, each cat received fewer than ten bilateral injections over a period of 3 months (Table 1). Within 5 min of the injections, after a rapid assessment of behaviour, posture and reflexes, the cats were put in the recording cage, where the behavioural observation continued by way of a TV camera. The recording sessions lasted for at least 4 h. The polygraphic records (paper speed 5 mm/s) were scored in 30-s epochs for waking, SS, and DS, according to standard criteria [22]. Waking was defined by a low-voltage, high-frequency or intermediate-frequency EEG activity (W1), or by a 4- to 8-Hz intermediate- to high-voltage EEG activity over the posterior lateral cortex in 50% or more of the scoring epoch (W2), associated with a well-sustained EMG activity. SS was defined by at least three 11- to 16-Hz sleep spindles per 30-s record from sigmoid cortex recordings, with less (SS1) or more (SS2) than 50% slow waves at 1–4 Hz, 50 µV or higher per 30-s record from posterior lateral cortex recordings, associated with variable, usually tonic EMG activity. DS was defined by a low-voltage, high-frequency EEG activity, no tonic EMG activity but occasional muscle twitches, bursts of rapid eye movements, PGO waves in the lateral geniculate nucleus and a continuous high-voltage theta rhythm (4–7 Hz) in the hippocampus. Although all five stages were scored, W1+W2 and SS1+SS2 were cumulated for statistical analysis. The following variables were calculated from the polygraphic recordings: (a) the percentage of the recording time spent in waking, SS, and DS, both cumulatively during the 4-h period and separately for each hour; (b) latency to DS onset; (c) mean number and duration of DS episodes; (d) duration of the longest DS episode; (e) ratio between DS and SS. Statistical analysis was performed with a two-tailed Student's *t*-test for the percentages of different sleep stages and with the non-parametric Mann-Whitney *U*-test for the rest of the data. In the analysis of the remaining results (site dependence, symmetrical injections) only values lying outside the 95% confidence interval for the controls were considered to be statistically significant.

Before sacrifice, 0.25 µl saline stained with 5% pontamine sky blue (Gurr BDH Chemicals, Poole, England) was injected bilaterally at the stereotaxic coordinates where methoxamine and prazosin had elicited the strongest effects on sleep. The cats were then deeply anaesthetized with pentobarbital (50 mg/kg, i.p.); their brains were removed, fixed in 10% formalin and cut into 50-µm sagittal sections. In four cases coronal sections were prepared. Serial frozen sections of the brainstem were mounted on glass slides and stained with neutral red to localize each cannula penetration and the extent of pontamine diffusion exactly. Every section showing traces of either cannula penetration or pontamine diffusion was visually projected, enlarged and drawn according to the plates of Bergman [2].

Table 1. Average number of injections, control recordings, and time intervals between injections and recordings for each cat[a]

Parameter	Values ± SEM
Number of cats	10
Average survival time after surgery (days)	93.7 ± 3.3
Average number of control recordings (C)	12.5 ± 0.2
Average number of injections (I)	8.7 ± 0.3
Average time interval between two I (days)	8.7 ± 0.3
Average time interval between C and I (days)	2.9 ± 0.2
Average time interval between I and C (days)	5.7 ± 0.2

[a] The values are means ± SEM and refer to ten different cats. For each cat the survival time was calculated from the day of surgery to the day of sacrifice. On the average, each cat wat recorded for control both before and after each injection

Results

Methoxamine

After bilateral infusion into the DPT of the α_1-agonist methoxamine, the cats remained quiet and reacted normally to various stimuli. No changes in posture and locomotion were observed: in particular, the extensor muscular tone of the forelimbs was unchanged and the placing reaction was normal. Vegetative reactions (such as salivation, panting, vomiting) were absent. Table 2 illustrates how the bilateral injection of methoxamine at the stereotaxic coordinates of P 1.0 to 3.0, LR 2.5 to 2.8, H −2.0 to −3.0 (4 µg in 0.25 µl saline, $n = 11$ experiments

Table 2. Effects of sleep/waking parameters of methoxamine infused bilaterally and symmetrically into the dorsal pontine tegmentum (4 µg in 0.25 µl saline)[a]

Parameter	Controls ($n = 60$)	Methoxamine ($n = 11$)
W (% of recorded time)	48.7 ± 4.0	60.3 ± 3.6*
SS (% of recorded time)	36.0 ± 3.2	35.4 ± 3.6
DS (% of recorded time)	15.3 ± 1.1	4.3 ± 0.6***
DS (number of episodes)	9.0 ± 0.9	4.0 ± 0.6***
DS latency (min)	38.9 ± 6.2	58.6 ± 16.2
DS mean duration (min)	4.3 ± 0.4	2.6 ± 0.5*
DS max. duration (min)	9.5	4.6***
DS/SS ratio	0.44 ± 0.03	0.14 ± 0.03***

[a] Controls include baseline recordings, saline infusions and simulated saline infusions. The values are means ± SEM and refer to recording sessions of 4 h. Student's *t*-test was used for the percentages of sleep stages, the Mann-Whitney *U*-test for the rest of the data. *n*, the number of experiments; W, waking; SS, synchronized sleep; DS, desynchronized sleep

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

in seven different cats) modified sleep/waking parameters. DS was strongly reduced, dropping from 15.3% of the total recording time in the controls to 4.3% after methoxamine. The decrease in the percentage of DS was due to the reduction in the number, the mean, and the maximal duration of DS episodes (see also Figs. 1–3). In one case methoxamine produced a total suppression of DS during the 4-h period. The waking time increased from 48.7% to 60.3%, while SS was not reduced, so that the ratio between DS and SS decreased from 0.44 in the controls to 0.14 after methoxamine.

Decreasing doses of methoxamine were injected into the same spot of the DPT of one animal, at intervals of 8 days, to study the dose dependence of the effect. As shown in Fig. 4, a dose of 4 µg in 0.25 µl reduced DS from 18.1% to 5.0%, while doses of 2 µg and 1 µg in the same volume (one trial per dose) were less effective, producing a 10.4% and 16.8% DS, respectively. Receptor desensitization could be ruled out, since a subsequent injection of the standard dose of methoxamine (4 µg in 0.25 µl) produced 4.7% DS. The effect of methoxamine was strictly site-specific because after bilateral injection 1–2 mm away from the critical region in the rostro-dorsal or caudo-ventral direction the percentage of DS remained unmodified.

Histological controls made at the end of the experiments demonstrated that the sites where methoxamine was effective corresponded to a limited region of DPT, at the stereotaxic coordinates of P 1.0 to 3.0, LR 2.5 to 2.8, H −2.0 to −3.0, which included the peri-LCa and the neighbouring dorsal pontine reticular formation, and extended from the ventral aspect of the brachium conjunctivum to the lateral tegmental field (Figs. 5 and 6). On the other hand, a normal percentage of DS was observed after bilateral injection into the gigantocellular tegmental field (P 3.0, LR 2.5, H −6.0, $n = 4$ experiments). In three cats a bilateral injection of carbachol (0.1 µg in 0.25 µl saline) in the same area where methoxamine was effective showed this spot to be cholinosensitive (Fig. 7).

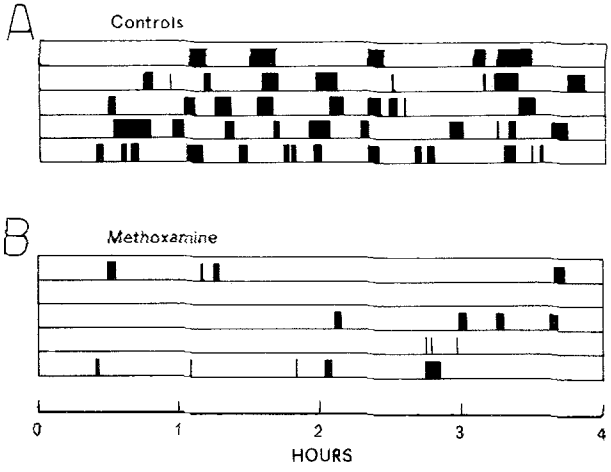

Fig. 2A, B. Distribution of DS episodes on a trial by trial basis in ten recording sessions in five cats. Each *horizontal strip* represents one recording session of 4 h. *Solid black bars* indicate the occurrence and duration of DS episodes. **A** Controls; **B** after the injection of methoxamine (4 µg in 0.25 µl saline). All injections were located bilaterally and symmetrically in the DPT at P 1.0 to 3.0, LR 2.5 to 2.8, H −2.0 to −3.0

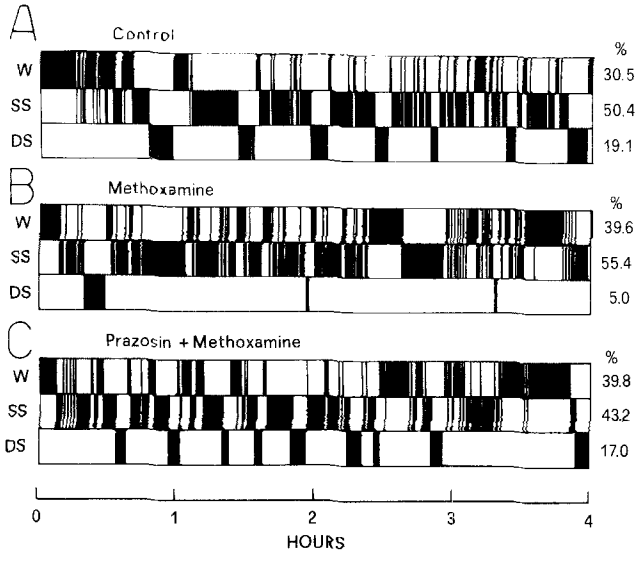

Fig. 1A–C. Hypnograms of three individual recording sessions from one representative cat (*cat 6*). **A** Control; **B** after injection of methoxamine (4 µg in 0.25 µl saline); **C** after prazosin injection (0.25 µg in 0.25 µl solvent) followed, 10 min afterwards, by methoxamine injection (4 µg in 0.25 µl saline). The injections were located bilaterally and symmetrically in the same spot of the dorsal pontine tegmentum (DPT; P 2.0, LR 2.5, H −2.0). The percentage of time spent in the different sleep/waking states is indicated on the *right side*. *W*, waking; *SS*, synchronized sleep; *DS*, desynchronized sleep

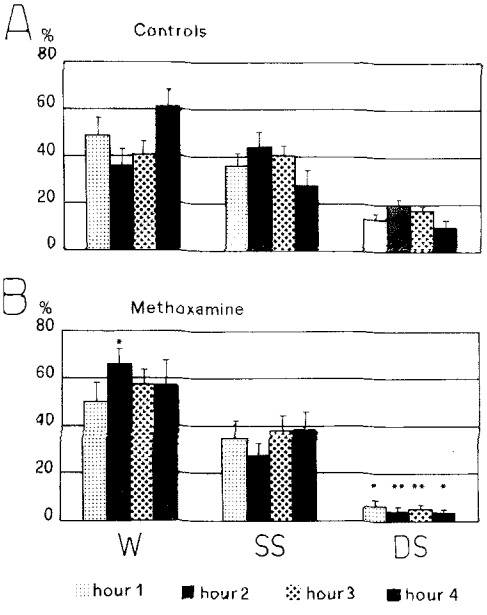

Fig. 3A, B. Mean percentage of time per hour spent in waking (*W*), synchronized sleep (*SS*), and desynchronized sleep (*DS*). **A** Controls; **B** after the injection of methoxamine (4 µg in 0.25 µl saline, $n = 11$ experiments). Each *bar* represents the mean ± SEM of seven different cats. All injections were located bilaterally and symmetrically in the DPT at P 1.0 to 3.0, LR 2.5 to 2.8, H −2.0 to −3.0. Cumulative 4-h data are shown in Table 2. $^*P < 0.05$; $^{**}P < 0.001$; *t*-test

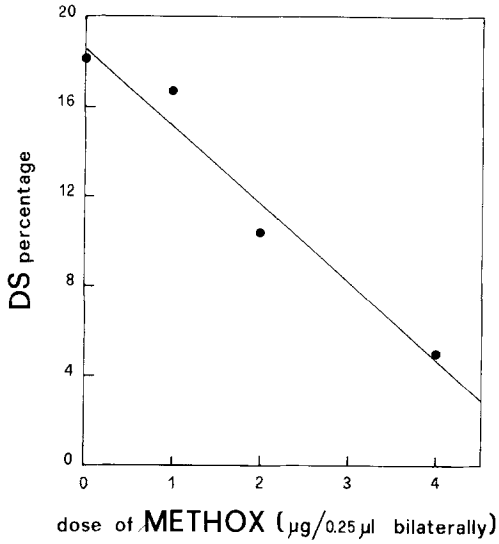

Fig. 4. Relationship between DS percentage and dose of methoxamine (*METHOX*). Three different doses of methoxamine (1, 2 and 4 μg in 0.25 μl saline bilaterally, one trial per dose), injected in the DPT of one cat (cat 6), in the same spot but in different experimental sessions, produced a graded reduction of DS (16,8%, 10.4%, and 5.0% of the total recording time respectively; control = 18.1%). Correlation coefficient = −0.99

Prazosin

Bilateral injections of the α_1-antagonist prazosin were performed in the same spots used for methoxamine (cf. Fig. 5). Preliminary tests with *N, N*-dimethylacetamide, the solvent of prazosin, ruled out any modifications of sleep/waking parameters as a result of this substance.

Table 3. Effects of sleep/waking parameters of prazosin infused bilaterally and symmetrically into the dorsal pontine tegmentum (0.025 or 0.0125 μg in 0.25 μl solvent)[a]

Parameter	Controls ($n = 32$)	Prazosin ($n = 6$)
W (% of recorded time)	50.4 ± 5.0	41.7 ± 6.9
SS (% of recorded time)	33.6 ± 4.9	39.3 ± 4.4
DS (% of recorded time)	16.0 ± 1.2	19.1 ± 3.0
DS (number of episodes)	8.5 ± 1.5	10.8 ± 3.1
DS latency (min)	46.4 ± 6.6	29.3 ± 5.1
DS mean duration (min)	4.9 ± 0.6	5.2 ± 0.8
DS max. duration (min)	10.2	9.6
DS/SS ratio	0.48 ± 0.04	0.48 ± 0.05

[a] Controls include baseline recordings, saline infusions and simulated saline infusions. The values are means ± SEM and refer to recording sessions of 4 h. Student's *t*-test was used to compare the percentages of sleep stages, the Mann-Whitney *U*-test for the rest of the data. *n*, the number of experiments. Differences between mean values were not significant

The injection of prazosin did not produce any evident alteration of behaviour, posture, and locomotion. In particular, no sedation was observed. Several concentrations of prazosin were used. We injected prazosin at relatively low doses (0.025 μg or 0.0125 μg in 0.25 μl solvent) in the sites where methoxamine had produced significant changes in DS parameters, corresponding to the peri-LCa and the neighbouring dorsal pontine reticular formation (P 1.0 to 3.0, LR 2.5 to 2.8, H −2.0 to −3.0). In this case, however, no statistically significant change in the measured variables was observed with respect to control recordings (Table 3, three animals, $n = 6$ experiments). This conclusion did not change when the two doses (0.025 μg

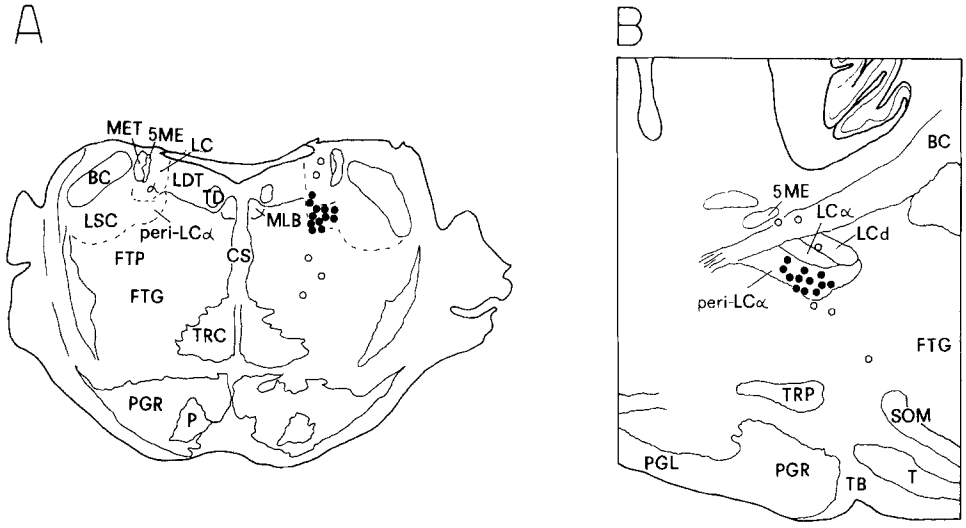

Fig. 5 A, B. Anatomical localization of injection sites in the DPT. **A** Coronal section of the brainstem at P 3.1, illustrating the location of effective and ineffective spots. **B** Sagittal section of the same area at the stereotaxic laterality of 2.5 mm. ●, Injection sites where methoxamine (4 μg in 0.25 μl saline, bilaterally, $n = 11$ experiments in seven different cats) reduced the number and duration of the episodes of DS. ○, The ineffective sites ($n = 10$ experiments). The same spots were also tested with prazosin injections, but no significant modification of DS was observed. *BC*, brachium conjunctivum; *CS*, superior central nucleus; *FTG*, gigantocellular tegmental field; *FTP*, paralemniscal tegmental field; *LCd*, locus coeruleus, pars dorsalis; *LCα*, locus coeruleus α; *peri-LCa*, peri-locus coeruleus α; *LDT*, laterodorsal tegmental nucleus; *LSC*, locus subcoeruleus; *MET*, mesencephalic trigeminal tract; *MLB*, medial longitudinal bundle; *P*, pyramidal tract; *PGL*, pontine grey, lateral division; *PGR*, pontine grey, rostroventral division; *SOM*, medial nucleus of the superior olive; *T*, nucleus of the trapezoid body; *TB*, trapezoid body; *TD*, dorsal tegmental nucleus; *TRC*, tegmental reticular nucleus, central division; *TRP*, tegmental reticular nucleus, pericentral division; *5ME*, mesencephalic trigeminal nucleus

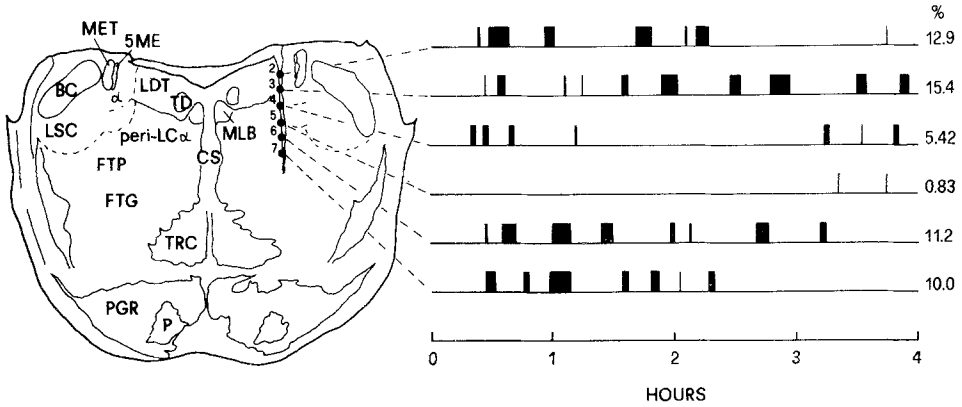

Fig. 6. Site specificity of methoxamine effects. Coronal section of the brainstem at P 3.1, showing the effects of local injection of the same dose of methoxamine (4 µg in 0.25 µl saline, bilaterally) in various pontine regions from dorsal to ventral sites. All six injections were performed in the same animal (cat 4) but in different experimental sessions (one trial per region). The percentage of time spent in DS is indicated on the *right side* (percentage DS in control recording = 16.3). *2–7*, Steps of comparable length at which the tip of the cannula was positioned. Each *horizontal strip* on the *right* represents the recording session of 4 h after the injection of methoxamine. *Solid black bars*, the occurrence and duration of DS episodes. Note that methoxamine was effective only when injected at depths *4* and *5* of the scheme, corresponding to stereotaxic coordinates of H −2.0 to −3.0. For abbreviations see Fig. 5

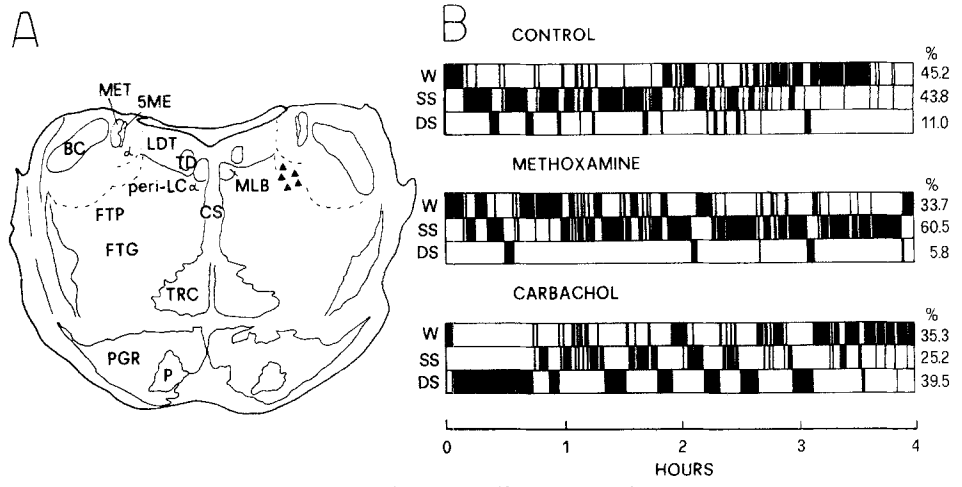

Fig. 7A, B. The sites where methoxamine was effective are also cholinoceptive. **A** Coronal section of the brainstem at P 3.1. ▲, Injection sites where methoxamine (4 µg in 0.25 µl saline, bilaterally, $n = 4$ experiments in three different animals) and carbachol (0.1 µg in 0.25 µl saline, bilaterally, $n = 3$ experiments in the same animals where methoxamine was injected) were effective in reducing and increasing DS, respectively. **B** Hypnograms of three representative recording sessions from one cat (cat 4) in the control, after the injection of methoxamine (4 µg in 0.25 µl saline) and after the injection of carbachol (0.1 µg in 0.25 µl saline). The percentage of time spent during the different sleep/waking states is indicated on the *right*. *W*, waking; *SS*, synchronized sleep; *DS*, desynchronized sleep. For abbreviations see Fig. 5

Table 4. Effects of pretreatment with prazosin on the methoxamine-induced changes of the sleep/waking parameters (0.25 µg prazosin in 0.25 µl solvent, followed, 10 min afterwards, by 4 µg methoxamine in 0.25 µl saline)[a]

Parameter	Controls ($n = 28$)	Prazosin + methoxamine ($n = 3$)	Methoxamine ($n = 6$)
W (% or recorded time)	28.3 ±5.3	30.8 ±5.7	56.3 ± 6.7*
SS (% of recorded time)	50.7 ±4.4	51.5 ±4.3	40.0 ± 6.3
DS (% of recorded time)	20.5 ±1.3	17.4 ±2.3	3.6 ± 1.0***
DS (number of episodes)	9.2 ±0.3	11.0 ±0.6*	3.4 ± 1.2**
DS latency (min)	33.3 ±4.5	31.5 ±2.5	66.0 ±43.5
DS mean duration (min)	5.3 ±0.3	3.8 ±0.6*	2.6 ± 1.1
DS max. duration (min)	10.2	7.6*	4.6
DS/SS ratio	0.40 ±0.03	0.34±0.04	0.09±0.03**

[a] Controls include baseline recordings, saline infusions and simulated saline infusions. The values are means ± SEM and refer to recording sessions of 4 h. Student's *t*-test was used for the percentages of sleep stages, the Mann-Whitney *U*-test for the rest of the data. $*P<0.05$; $**P<0.01$; $***P<0.001$. *P* values for prazosin + methoxamine are for comparison with the controls, those for methoxamine are for comparison with prazosin + methoxamine. *n*, the number of experiments

and 0.0125 µg in 0.25 µl solvent) were considered separately. At higher doses (2.5 µg and 0.25 µg in 0.25 µl solvent, $n = 11$ experiments, five animals) the injection of prazosin produced inhomogeneous and statistically inconsistent effects. Dependig upon the individual animal or experiment, the amount of DS could show a moderate increase, a decrease, or no change with respect to control recordings.

On the other hand, prazosin was consistently able to prevent the effects of methoxamine when injected before the α_1-agonist (prazosin 0.25 µg in 0.25 µl solvent, followed, 10 min afterwards, by methoxamine, 4 µg in 0.25 µl saline, $n = 3$ experiments in three different cats, Fig. 1 C; Table 4). As shown in Table 4, the percentage of time spent in waking, SS, and DS after prazosin + methoxamine was not significantly modified. Note, however, that the increase of number of DS episodes in cats treated with both drugs was associated with a decrease in their mean and maximal duration, so that the total time spent in DS was similar to that of the controls.

Discussion

This study demonstrates that the α_1-agonist methoxamine, when microinjected bilaterally into the DPT, produced a clear-cut reduction of DS. The decrease of the percentage of DS over the total recording time was due both to an increase in DS latency and to a reduction in number and duration of DS episodes. This effect was dose-dependent and site-specific, in that methoxamine was effective only when injected into the peri-LCa and into the pontine reticular formation immediately ventral to it. This is the same region where the infusion of other noradrenergic agents influenced the amount of DS [20, 21]. This is also the region where cholinergic agonists can optimally trigger a sustained increase of paradoxical sleep [23, 24], as we confirmed by testing the effective spots with carbachol injections.

Since after methoxamine there was a moderate increase of waking time without a decrease in the percentage of SS, we can reasonably exclude the possibility that DS reduction was due to an aspecific disturbing effect of the drug on the sleep/waking cycle. Moreover, no important changes in blood pressure, heart rate [18], and brain temperature [19] have been observed after systemic injection of this α_1-agonist. The presence or absence of α_1-adrenoceptors on cerebral microvessels has not been established unequivocally (see [9] for references). However, the site specificity of the suppression of DS suggests that the action of methoxamine did not depend on the induction of vascular changes. Finally, the dose dependence of the reduction of DS, and the fact that it could be prevented by pretreatment with the α_1-antagonist prazosin indicate that the action of methoxamine was specifically mediated by α_1-adrenoceptors.

The observed reduction of DS is in agreement with the results of systemic injection experiments. Methoxamine acts as a central excitant causing an arousal reaction when infused i.p. or i.v. in several animal species (see [19] for references). In rats [18], cats [6], and dogs [19] methoxamine reduced the percentage of total sleep and DS in a dose-dependent manner. Moreover, methoxamine has been described as improving cataplexy in canine narcolepsy (see [16] for references), and some α_1-adrenoceptor agonists such as Modafinil have been successfully employed in narcoleptic and hypersomniac humans [10].

α_1-Adrenoceptor binding sites have been demonstrated in the rat brainstem [9]. High binding levels have been found in the DPT, particularly in the LC region and peribrachial area, while the levels in more ventral regions such as the gigantocellular tegmental field and the paralemniscal tegmental field are much lower. The question arises of how the activation of α_1-adrenergic receptors produces a reduction of DS. Methoxamine could act by activating noradrenergic LC neurons. However, microinjections into the dorsal part of the LC, where most noradrenaline-containing neurons are found, were always ineffective. In addition, Chamba et al. [3] found no correlation between the distribution of noradrenaline-containing cells and α_1-receptor binding, suggesting that these receptors may be located primarily on non-noradrenergic neurons. Another possibility is that methoxamine acted by directly affecting DS-on cells responsible for the execution of DS. In general, however, α_1-adrenoceptors tend to produce excitation of postsynaptic cells, which in this case would lead to an effect on DS opposite to the one observed. It is thus possible that the effect of methoxamine on DS is not based on a direct action on DS-on cells, but that additional populations of cells may be implicated. For instance, many GABAergic interneurons have been identified in the DPT and it has been hypothesized that they may mediate some interactions between DS-on and DS-off cells [8].

The results obtained with prazosin are difficult to evaluate. At low doses, prazosin was ineffective in modifying sleep/waking variables. At higher doses, prazosin produced inconsistent effects, in some cases a decrease in DS, in others an increase, and in some experiments no change at all. On the other hand, prazosin was consistently able to prevent the DS-suppressive effect of methoxamine, as indicated by the fact that, in cats treated with both drugs the percentages of waking, SS, and DS were similar to those in the controls. Systemic injection experiments with prazosin have also yielded contrasting results, depending on the animal species and on the dose [1, 6, 11, 13, 15, 18, 25]. For instance, Hilakivi and Leppävuori obtained an increase of DS after systemic injections of prazosin in the cat at low doses (0.5 – 3 mg/kg), but not at higher doses (10 mg/kg), where an initial depression of DS was instead observed [6]. In the rat one study has shown a reduction of DS within a wide dose range [18], and another study [13] has demonstrated a short-term (2 – 3 h) reduction of DS followed by a significant increase. Variable results have been described with other α_1-adrenergic antagonists, such as thymoxamine and fenossibenzamine [5, 14]. It has been suggested that at high doses the action of prazosin on sleep mechanisms is masked by some cardiovascular or metabolic effects, such as vasodilatation, decrease of blood pressure, hypothermia and increase in noradrenaline turnover (see [6] for references), although such confounding factors are less

likely to play a significant role in the case of local microinjections.

In summary, the present results indicate that α_1-adrenoceptors are involved in the control of the activity of DS-generating circuits in the brainstem. Such involvement was quite evident when an agonist such as methoxamine was employed, but could not be assessed with an antagonist such as prazosin. This suggests that, at least in the freely moving unanaesthetized cat, the α_1-noradrenergic tone in the DPT is rather low. These findings complement previous data from our laboratory, which showed that the β-agonist isoproterenol and the α_2-agonist clonidine, when locally infused in the DPT, respectively reduced or completely suppressed DS [20, 21]. On the other hand, unlike the α_1-antagonist prazosin, the β-antagonist propranolol was able to enhance the percentage of DS. Taken together, this evidence indicates that noradrenaline can inhibit brainstem mechanisms of DS by acting through α_1-, α_2-, as well as β-receptors, and it is thus consistent with the general predictions of the reciprocal interaction model of DS generation [7]. Depending upon the specific receptor type, however, different mechanisms and different populations of units in the DPT may be directly responsible for such inhibition.

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