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ROLE OF THE LOCUS COERULEUS IN THE CONTROL OF PARADOXICAL SLEEP GENERATION IN THE CAT

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INTRODUCTION

Following the discovery of rapid eye movement (REM) sleep in humans by Aserinsky and Kleitman (1), a similar sleep state has been identified in the cat by Dement (6) and Jouvet and his colleagues (14), and called desynchronized or paradoxical sleep (PS). Using brain lesion and transection techniques, Jouvet showed, as early as 1962, that a state nearly identical to PS could be observed even after removal of all neural tissues above the level of the rostral pons and that the pons is the most critical region in the brain for PS generation (11). Jouvet further proposed that the activity of noradrenergic neurons in the nucleus locus coeruleus (LC; A6 cell group (5)) of the dorsolateral pontine tegmentum is critical for the generation of PS and its signs (12, 13). Subsequent studies revealed, however, that noradrenergic LC neurons cease firing just prior to and during PS (see ref. (9) for review), leading to the postulate that LC noradrenergic activity plays a critical inhibitory, instead of an executive, role in PS generation by inhibiting PS-executive or PS-on neurons located in the peri-LC region of the pons (8, 15, 16). The exact role of noradrenergic LC neurons in the tonic control of PS generation, however, still remains to be determined. In the present study, therefore, we have examined *in vivo* effects on behavioral states of microdialysis application to the LC of clonidine, a selective and potent agonist of α_2 adrenoceptors, activation of which causes cessation of discharge of noradrenergic LC neurons.

MATERIAL AND METHODS

A total of 9 adult cats were implanted with electrodes for polygraphic recordings and guide cannulae for microdialysis application of drugs, as previously described (3). For extracellular single unit recordings, one bundle of 6 flexible, Formvar-coated, stainless steel wires (32 μm in diameter) was inserted into the LC through the guide cannulae (24 gauge) of a mechanical microdrive assembly. For inverse microdialysis, one guide cannula (23 gauge) was incorporated into the microdrive assembly between the two microelectrode bundles (1 mm separation between the centers of the guide cannulae), as previously described (19). Drugs were dissolved in Ringer's solution and administrated continuously through a microdialysis probe (1 mm in length and 0.23 mm in outer diameter: Eicom A-L-50-01) during two consecutive hours. This experimental period was preceded by at least 1-h control recording session and followed by 4-6 h recordings to assess

recovery by applying Ringer's solution alone. In order to avoid any effects of acute tissue damage on the EEG and behavioral states, each probe was inserted through a guide cannula on the day preceding each experimental session. The probe was left in the brain for 5-7 days and drugs were applied on experimental days 2-5. Effects of drugs were analyzed by comparing data from each experimental group with those from paired control recordings using the same animal, site and experimental period by means of two-tailed t-test. States of vigilance were scored minute by minute and classified according to standard criteria into the four phases of: (i) wakefulness (W); (ii) light slow-wave sleep (S1); (iii) deep slow-wave sleep (S2); and (iv) PS. At the end of experiments, several unit recording sites were coagulated by passing a cathodal current under deep anesthesia. The localization of the tip and tracts of microelectrodes, as well as microdialysis probes, was determined histologically, as described previously (18).

RESULTS

Feline LC (A6 cell group (5)) consists of two components: i) principal LC or LC proper (LCp), located in the periaqueductal grey and ii) locus coeruleus pars alpha (LCα), found in the reticular formation just ventral to the LCp (17). As illustrated in Figure 1A, the microdialysis probes were applied to either the LCp or the caudal part of the LCα. These regions of the LC complex contain a high density of noradrenergic neurons, and are virtually devoid of cholinergic neurons (Fig. 1B).

Effect of microdialysis application of clonidine on noradrenergic LC neuronal activity

We have first examined the extent of drug diffusion within the LC. For this purpose, we carried out single-unit extracellular recordings in the rostral and caudal regions of the LCp and LCα and applied clonidine by microdialysis infusion to the middle region. We have recorded a total of 59 neurons that were classified as noradrenergic on the basis of previously established criteria (9), namely: *i)* an action potential of long duration, *ii)* slow, irregular discharge activity during quiet waking (mean ± s.d. = 0.43 ± 0.29 spikes/s), *iii)* complete suppression of firing during PS, and iv) histological localization of recording sites to the LC. The effect of microdialysis application of the α_2-adrenoceptor agonist, clonidine (50 or 200 µM), was tested on 15 presumed noradrenergic LCp and LCα neurons. As shown in Figure 2, when clonidine was applied to near (< 1 mm) the unit recording site, all cells exhibited state-independent depression of spontaneous discharge. This inhibition of discharge occurred within 5-10 min after the start of drug application and lasted for 30-60 min or more after the end of application, depending on the duration of drug application. The results indicate that the applied clonidine is able to affect a large population of presumed noradrenergic LC neurons located near the drug application site.

Effect of microdialysis application of clonidine on wake-sleep states

As shown in Figure 3, unilateral application of 50 or 200 µM clonidine, which causes cessation of discharge of a large population of noradrenergic LC neurons, had no significant effect on PS amount, when applied to either periaqueductal (LCp) or reticular (LCα) component of the LC complex. Similarly, no significant change in

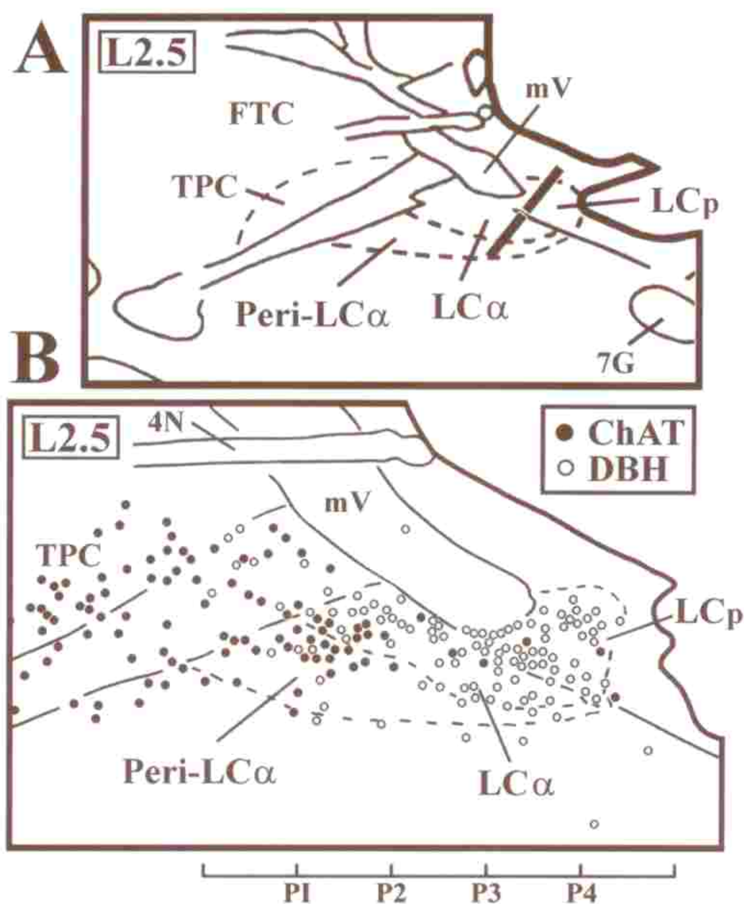

Fig. 1. *Diagram of a sagittal section at L2.5 of the cat brainstem showing (A) drug applications sites and (B) localization of noradrenergic (open circles) and cholinergic (closed circles) neurons revealed by dopamine-betha-hydroxylase (DBH) and choline acetyltransferase (ChAT) immunohistochemistry, respectively.*

The black strips indicate the location of the microdialysis probe membranes that were placed in the LCp or LCα at an angle of 52° from the horizontal. 4N, trochlear nerve; 6, abducens nucleus; 7G, genu of the facial nucleus; BC, brachium conjunctivum; FTC, central tegmental field; mV, mesencephalic tract of trigeminus; peri-LCα; peri-locus coeruleus alpha; TPC, cholinergic part of the nucleus tegmenti pedunculopontinus pars compacta.

PS architecture, such as PS latency, duration and frequency, was seen during the 2h drug application period (Tab. I). At the concentration of 50 μM, however, applied clonidine to the LC induced a significant decrease in W and increase in S2: the amount of W decreased from 23.8 ± 3.8 (n = 6) in the baseline to 9.5 ± 3.4 (n = 6) during the 2h drug application period, while S2 increased from 32.0 ± 2.5 min in the baseline to 44.9 ± 5.2 min during the drug application period.

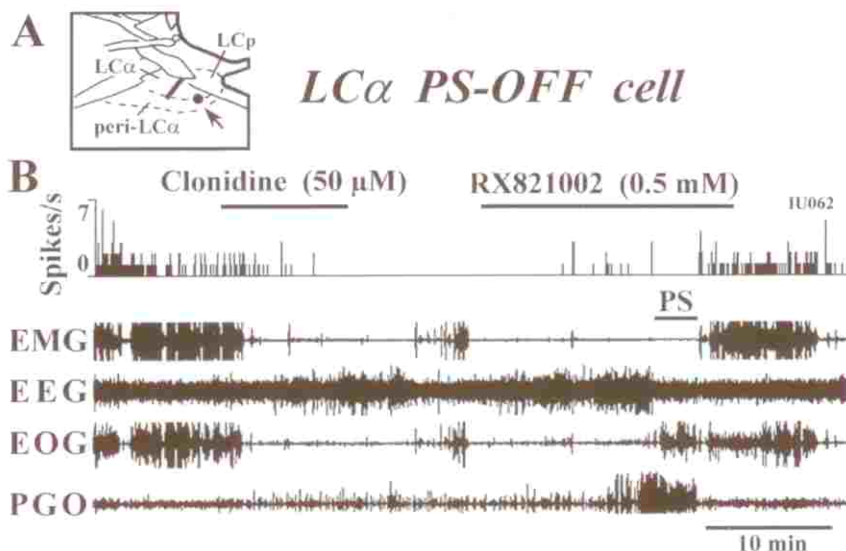

Fig. 2. *Drawing of a sagittal section (A) and polygraph traces (B) showing the effect of application of clonidine and RX821002 on a presumed noradrenergic neuron recorded in the LCα.*

The closed strip and circle in A indicate the localization of the microdialysis probe membrane and the unit recording site, respectively. The horizontal bars indicate the drug application periods. Note the suppression of unit discharge by clonidine and the reversal of the unit depression by RX821002, an α_2-selective adrenoceptor antagonist.

DISCUSSION AND CONCLUSION

In the present study, pharmacological manipulation of noradrenergic LC neurons failed to affect PS generation. Applied clonidine induced complete and long-lasting suppression of noradrenergic LC neuronal activity near the drug application site, but the same application had no effect on PS, at concentrations of 50 or 200 μM, though 50 μM clonidine induced a significant decrease in W and increase in S2 following the application to the LCp. Our findings, therefore, are in general agreement with those from early brain lesion studies showing that destruction of noradrenergic LC neurons has no effect on PS generation (22, 24).

The probe membrane that we have used was 1 mm in length and had an external diameter of 0.23 mm. This small size of the membrane permits us to minimize the diffusion of the drug delivered outside the target zone. The perfusate has been reported to diffuse mainly laterally from the membrane (3). In the present study, applied clonidine induced, even at the low concentration, complete and long-lasting suppression of presumed noradrenergic LC neurons near (< 1.0 mm) the drug application site (Fig. 2). This finding strongly suggests that low concentrations (< 50 μM) of clonidine are sufficient to depress a large or even entire population of noradrenergic target neurons and that high concentrations of clonidine may affect neurons

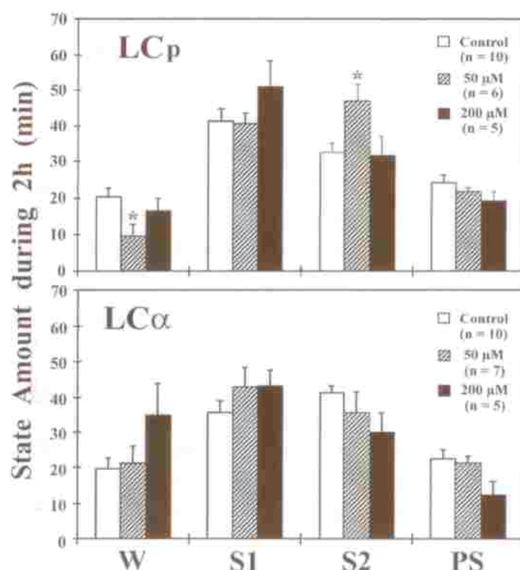

Fig. 3. *Effects on wake-sleep states of microdialysis application of clonidine to the LCp and LCα.*

The values are the mean time (min) ± SEM spent in W, SI, S2 or PS during 2h perfusion with 50 or 200 µM clonidine. 'Control' indicates the mean baseline values from all paired controls. The effects of clonidine were analyzed for each state using the two-tailed t-test and comparing the experimental values with those of paired controls obtained from the same animal, site and experimental period. $*P < 0.05$, compared with paired controls.

Tab. I. Effects of application of clonidine to the LCp and LCα on latency, duration and frequency of PS.

Site	LCp			LCα	
Condition	Baseline	Clonidine			
	(Ringer's) (n = 13)	50 µM (n = 6)	200 µM (n = 5)	50 µM (n = 7)	200 µM (n = 5)
Latency (min)	12.6 ± 2.6	18.8 ± 3.6	15.0 ± 6.9	13.6 ± 4.2	28.2 ± 9.6
Duration (min)	5.6 ± 0.5	4.5 ± 0.4	4.1 ± 0.6	5.2 ± 0.8	3.4 ± 0.6
Number (n)	4.3 ± 0.4	5.0 ± 0.4	5.0 ± 0.9	4.3 ± 0.4	3.4 ± 0.8

The values are the mean time or number (± SEM) observed during 2h application to the LCp and LCα of Ringer's solution (baseline), 50 µM and 200 µM clonidine. The number of trials is shown in parentheses. "Baseline" indicates the mean values from all paired controls. PS latency is the time from the beginning of drug infusion (when the drug reach the microdialysis membrane) to the first PS episodes. Duration and number indicate, respectively, the mean duration and number of PS episodes during 2h drug application. The effects were analyzed by two-tailed Student's t-test by comparing the experimental values with those of paired controls obtained from the same animal, site and experimental period. The difference was not statistically significant.

outside the target zone. In the present study, when applied to the LCp, 50 μM clonidine produced a significant decrease in W and an increase in SWS without affecting PS. When applied to the LCα, 50 μM clonidine had no effect, whereas 200 μM clonidine tended to inhibit PS generation as shown by a non-significant decrease in PS amount, duration and frequency, and a non-significant increase in PS latency (Fig. 3; Tab. I). It appears that this PS-inhibiting effect is due to the diffusion of the drug to the peri-LCα located just medial to this target structure, since 50 and 200 μM clonidine had a significant and dose-dependent PS-inhibiting effect when directly applied to the peri-LCα (4). These findings again point to the crucial role played by the peri-LCα, but not the LC, in the executive mechanisms of PS generation (20).

Pharmacological depletion of catecholamines by inhibition of their synthesis causes an increase in both SWS and PS (10). The α_2 adrenergic agonist clonidine suppress PS or REM sleep in the cat (2, 4, 23) and in humans (7). A key question arises as to brain catecholaminergic structures exerting a critical inhibitory influence on the pontine PS-on neurons. The peri-LCα receives both adrenergic afferent projections from the medulla (C1 cell group) and noradrenergic projections from pontine (A4, A5, A6 and A7) and medullay (A1 and A2) cell groups. Pontine PS-on neurons are inhibited by iontophoretically applied epinephrine or norepinephrine (21). Direct application of epinephrine or norepinephrine to the peri-LCα results in PS inhibition (3), and this effect is mediated by α_2 adrenoceptors (2). It may be that medullay catecholaminergic neurons play a critical role in this inhibitory mechanisms of PS generation. The activity of the medullay neurons during the sleep-waking cycles, however, is not yet known and will be an important subject for future studies.

In conclusion, our present findings support the postulate that noradrenergic LC neurons play an important role in the control of W and SWS, but do not support the theory that LC noradrenergic activity inhibits PS-executive neurons located in the pons and that inactivation of noradrenergic LC neurons plays a critical role in the initiation and maintenance of PS.

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