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Increased REM sleep after intra-locus coeruleus nucleus microinjection of melanin-concentrating hormone (MCH) in the rat

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ABSTRACT

A study was carried out on the effects of unilateral microinjection of melanin-concentrating hormone (MCH) into the right locus coeruleus (LC) on the sleep–wake cycle in rats prepared for chronic sleep recordings. MCH 200 ng significantly augmented rapid-eye-movement sleep (REMS) time during the first, second and third 2-h of recording. Furthermore, MCH 100 ng induced a significant increase of REMS during the first 2-h period after treatment. The increment of the behavioral state was related to a greater number of REMS episodes.
It is suggested that MCH deactivation of noradrenergic neurons located in the LC facilitates the occurrence of REMS.

1. Introduction

Recent studies have shown that the neuropeptide melanin-concentrating hormone (MCH) participates in the regulation of the sleep–wake cycle (Monti et al., 2013; Torterolo et al., 2011). In this respect, MCHergic neurons localized to the lateral hypothalamus and incerto-hypothalamic area innervate the brain areas involved in the occurrence of wakefulness (W), non-rapid-eye movement sleep (NREMS) and rapid-eye-movement sleep (REMS) (Bittencourt and Elias, 1998; Hervieu et al., 2000). Among the structures innervated by the MCHergic system is the locus coeruleus (LC), a noradrenergic nucleus that plays a key role in waking-related activities (Berridge et al., 2012; Szavadi, 2013).

In the present study we explored the effects of microinjection of MCH into the right LC on sleep variables in the rat. The local administration of the neuropeptide into the LC produced a significant increase of the time spent in REMS while W and NREMS remained almost unchanged.

2. Materials and methods

2.1. Animals

Male Wistar rats ($n = 8$) weighing 320–350 g at the time of surgery were used. All experiments were conducted in accordance with the National Institutes of Health (USA) guidelines for the care and use of laboratory animals. All procedures were approved by the Institutional Animal Care Committee of the Medical School, Montevideo, Uruguay.

2.2. Surgical procedures

Details of the surgical procedure were described previously (Lagos et al., 2009). The animals were implanted chronically with electrodes in the frontal and occipital cortices and neck muscles for recording of electroencephalogram and electromyogram, respectively. Additionally, a guide cannula (26 gauge) was implanted 2 mm above the right LC at coordinates, AP −9.84; L 1.4, and V −7.0 (Paxinos and Watson, 2005). MCH (100 and 200 ng in 0.2 μl of sterile saline) or vehicle (0.2 μl of sterile saline) was injected into the right LC with an injection cannula (29 gauge) which extended 2 mm beyond the guide. *The right LC was selected at random.*

Histological verification of cannula/injection sites was carried out at the end of the experiments.

Abbreviations: BFB, basal forebrain; DRN, dorsal raphe nucleus; GABA, γ-aminobutyric acid; 5-HT, serotonin; LC, locus coeruleus nucleus; LDT/PPT, laterodorsal and pedunculopontine tegmental nuclei; LS, light sleep; MCH, melanin-concentrating hormone; NE, noradrenaline; NREMS, non-rapid-eye movement sleep; REMS, rapid-eye movement sleep; SLD, sublaterodorsal tegmental nucleus; SWS, slow wave sleep; W, wakefulness.

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2.3. Recording and sleep scoring

Ten days after implantation the animals were adapted to the recording and injection procedures. The rats were housed individually under a 12-h light/12-h dark cycle. MCH and control solution were always administered during the light phase. All records were scored for W, NREMS [light sleep *(LS)* and slow wave sleep (SWS)] and REMS. Latencies to SWS and to REMS, the mean number of REMS periods and the mean duration of REMS episodes were quantified also (Lagos et al., 2009).

2.4. Experimental design

Recording were started 15 min after MCH or control solution injection, and continued for 6 h. At least 3 days was allowed to elapse between experiments.

2.5. Statistics

All values are presented as mean ± S.E.M. The distribution of the samples was normal, which leads us to use a parametric test for statistical analysis. The experimental design was a within-subject design where statistical significance of the differences among groups (control, MCH 100 and 200 ng) was evaluated utilizing a one-way repeated measures ANOVA (GraphPad InStat). Post hoc comparisons was performed with Dunnett's multiple comparisons test when the ANOVA indicated significance ($P < 0.05$).

3. Results

Histological analysis of the injection sites showed that 7 of the 8 animals originally included in the study received microinjections of MCH that were confined within the limits of the right LC. A representative photomicrograph of a coronal section with the injection site in the right LC is shown in Fig. 1. The results obtained after recording sessions of 6 h following microinjections of MCH or vehicle into the LC are summarized in Table 1. Compared with the control vehicle MCH 200 ng significantly increased REMS from a control value of 27.7 ± 4.1 min (7.7% of the total recording time) to 39.6 ± 3.5 min (11.0% of the total recording time). The increment of REMS time was related to a greater number of REMS periods. SWS and REMS latency were not significantly modified (Table 1).

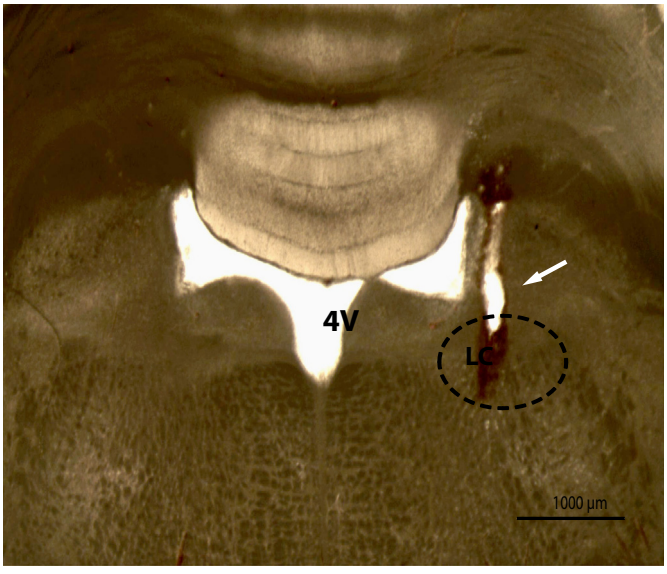

Fig. 1. Representative photomicrograph of a coronal section at the level of the right LC. The microinjection site is recognizable by the cannula track lesion. Abbreviations: LC, locus coeruleus nucleus; 4 V, fourth ventricle.

Table 1
Effects of MCH microinjected into the locus coeruleus nucleus on sleep and waking during 6-h polysomnographic recordings.

	Control	MCH (100 ng)	MCH (200 ng)
Wakefulness	49.6 ± 5.2	45.4 ± 4.2	41.9 ± 5.4
Light sleep	47.7 ± 4.7	43.4 ± 5.1	42.7 ± 3.9
Slow wave sleep	235.0 ± 8.0	239.9 ± 8.0	235.8 ± 8.9
REM sleep	27.7 ± 4.1	31.3 ± 4.7	39.6 ± 3.5*
Number of REM periods	14.1 ± 2.0	14.2 ± 1.3	18.6 ± 1.2*
Mean REM period duration	2.0 ± 0.1	2.1 ± 0.1	2.1 ± 0.1
Slow wave sleep latency	3.3 ± 1.4	3.7 ± 1.3	3.1 ± 1.6
REM sleep latency	48.6 ± 7.1	33.6 ± 7.1	37.4 ± 5.1

Sleep stages, mean REM period duration and sleep latencies were quantified in minutes. *All the values are the mean ± S.E.M. Seven animals were in each experimental group. The doses are in nanograms. The matching corresponding to wakefulness ($p = 0.05$), light sleep ($p = 0.001$) and slow wave sleep ($p = 0.02$) was effective, which allowed us to exclude a low statistical power.* *$p < 0.05$; significant statistical difference with respect to control (Dunnett's test). *MCH 200 ng significantly increased REM sleep duration and the number of REM periods over the 6-h recording period.*

Analysis of sleep variables in 2-h blocks showed that MCH 200 ng significantly augmented REMS during the first, second and third 2-h of recording. Additionally, MCH 100 ng induced a significant increase of REMS during the first 2-h period after treatment (Fig. 2). W, light sleep and SWS showed slight but inconsistent changes that did not attain significance (Table 1 and Fig. 2).

4. Discussion

The present study shows for the first time that microinjection of MCH into the LC during the light phase of the light–dark cycle causes a significant increase of REMS and of the number of REMS periods. The increase of REMS after MCH 200 ng administration attained significance during the first, second and third 2-h block of the recording period.

In the updated version of the reciprocal-interaction hypothesis of REMS generation (McCarley, 2007), cholinergic neurons of the *laterodorsal and pedunculopontine tegmental nuclei* (LDT/PPT) are identified as promoting REMS, and interact with serotonergic (5-HT) and NE neurons of the DRN and LC, respectively, that inhibit REMS. *γ-aminobutyric acid* (GABA)ergic mechanisms also play a role inactivating the neurotransmitter systems responsible for the inhibition of LDT/PPT cholinergic (REMS-on) neurons. This would lead to the activation of *sublaterodorsal nucleus* (SLD) glutamatergic neurons and the occurrence of REMS.

The revised model of REMS control proposed by Luppi et al. (2006) poses that during REMS the ventrolateral periaqueductal gray and the paragigantocellular reticular nucleus GABAergic REMS-on neurons inhibit REMS-off activity in the DRN, LC and lateral pontine tegmentum. As a result, SLD REMS-on glutamatergic activity is disinhibited. The authors have suggested also that inhibitory MCH connections to the REMS-off structures would participate in REMS expression (Luppi et al., 2013).

Thus, both models of REMS control propose that inhibition of LC noradrenergic neurons facilitates REMS occurrence.

With respect to the role of the noradrenergic system during the sleep–wake cycle, it has been reported that: 1. during quiet W NE neurons fire in a slow and regular fashion, while during active W the neuronal activity shows a significant increase. As the animal enters NREMS the mean discharge rate is progressively reduced, and during REMS there is a further decrease or even a cessation of neuronal activity; 2. dopamine-β-hydroxylase-deficient mice show an increase of REMS during the dark period. Similar effects on REMS have been found after systemic administration of the neurotoxin DSP-4 which selectively lesions the NE neurons; 3.

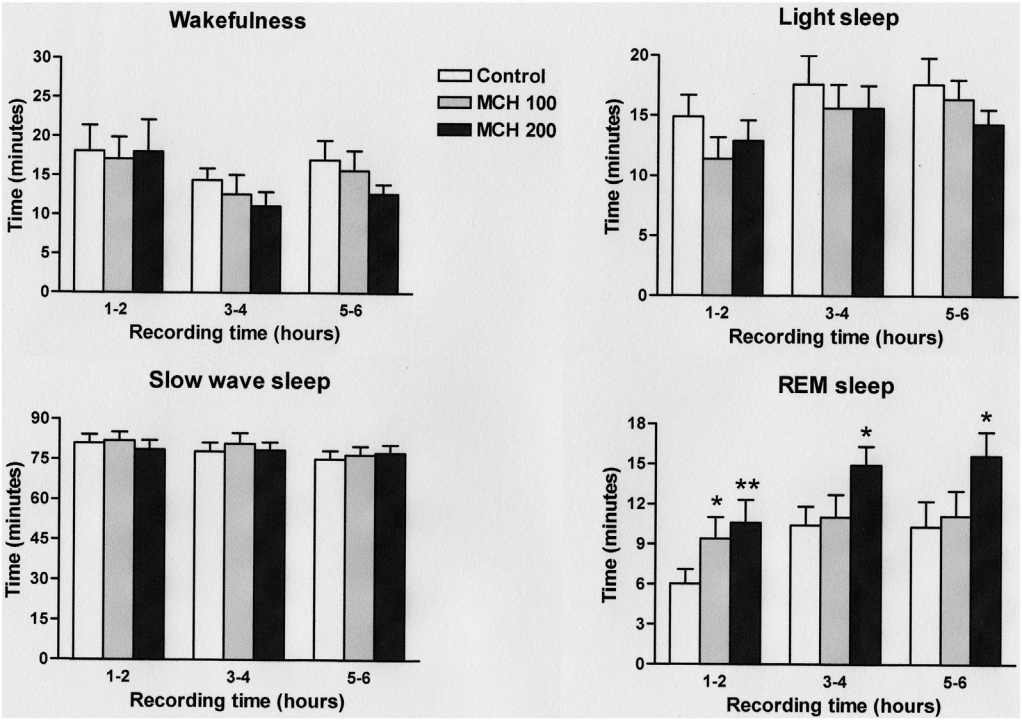

Fig. 2. Effects on sleep and W of MCH microinjected into the right LC. Ordinate: time spent in sleep and W (min ± S.E.M.). Abscissa: time after injection. *Seven animals were in each experimental group*. Compared with control values $^{*}P < 0.05$; $^{**}P < 0.01$ *(Dunnett's test)*. MCH 200 ng induced a significant increase of REMS during the first, second and third 2-h recording period. In addition, the 100 ng dose of MCH increased REMS during the first 2-h period after treatment.

systemic administration of the NE reuptake inhibitors amphetamine, cocaine and nisoxetine or the selective α receptor agonist methoxamine significantly increases W and reduces NREMS and REMS in the cat and the rat. Pretreatment with the α -receptor antagonist prazosin prevents the effects of methoxamine on sleep variables. Systemic injection of the β-adrenoceptor agonist clenbuterol does not significantly modify sleep variables in the rat, while the α_2-receptor agonist clonidine increases NREMS and suppresses REMS (for reviews see Berridge et al., 2012; Monti et al., 2013; Szavadi, 2013).

It can be proposed that the increased availability of NE in the central nervous system induces an increment of W and a reduction of NREMS and REMS, and this outcome would be related mainly to the activation of the α -receptor.

Harthoorn (2007); Bittencourt (2011) initially reported the existence of MCH terminals within the LC. More recently, Yoon and Lee (2013) examined the distribution to the LC of fibers from MCH neurons located within medial and lateral subdivisions of the lateral hypothalamus. The authors established that MCH projections to the nuclear core of the LC exhibit differential distribution with a predominance of the lateral subdivision over the medial one. It has been proposed, in addition, that MCH neurons might utilize GABA as a co-transmitter, since their soma contain the enzyme glutamic acid decarboxylase (Gao and van den Pol, 2001; Elias et al., 2001; Sapin et al., 2010). In support of the proposal, Del Cid-Pellitero and Jones (2012) found that a small number of MCH varicosities present at the LC in the rat was immunopositive for the vesicular transporter for GABA. Furthermore, the MCH varicosities containing the vesicular transporter for GABA contacted the tyrosine hydroxylase-immunostained neurons of the LC.

Thus, it can be suggested that under normal conditions MCH and to a smaller extent GABA, released by MCH-containing neurons would inhibit the noradrenergic cells located in the LC and increase REMS values. Our finding that local microinjection of MCH into the LC augments REMS time further supports the involvement of the neuropeptide in the regulation of the behavioral state.

Concerning the underlying pathophysiology of major depression, there are clinical data indicating alterations of 5-HT, NE and dopamine neurotransmission at central sites. The finding that a number of antidepressant drugs increase neurotransmission of 5-HT and/or NE through inhibition of their reuptake tends to support the proposal.

Preclinical findings tend to suggest that the hyperactivity of the MCHergic system could be involved in the pathophysiology of major depression. Accordingly, MCH administration into the rat DRN elicited prodepressive behaviors evaluated in the forced swimming test. The depressive-like response was prevented by pretreatment with the selective serotonin reuptake inhibitor fluoxetine. Opposite, immunoneutralization of MCH induced an antidepressant-like effect (Lagos et al., 2011). The induction of a depressive-like response was related to the activation of MCH-1 receptors, as the specific MCH-1 antagonist ATC0175 prevented this effect (Urbanavicius et al., 2014). Interestingly, microinjection of MCH into the DRN enhanced also REMS in the rat, a classical trait of major depression (Lagos et al., 2009). It could be tentatively proposed on the basis of preclinical findings, that depression in man would partly involve MCH reduction of serotonergic tone.

There are experimental evidence demonstrating that an increment in the noradrenergic tone augments climbing behavior in the forced swimming test in the rat (Cryan et al., 2002; Detke et al., 1995). Thus, the possibility exists that MCH microinjction into the LC would produce also a depressive effect. However, further studies are needed to determine possible alterations in the noradrenergic neurotransmission induced by MCH administration into the LC.

5. Conclusions

This study examined the involvement of the LC in the MCH regulation of sleep and W in the rat. Microinjection of MCH (100–200 ng) into the right LC during the light phase of the light–dark cycle increased REMS and the number of REMS periods.

Author contributions

All of the authors designed the experiments, collected data, performed analysis discussed the results and wrote the manuscript.

Conflict of interest statement

The authors declare that there are no conflicts of interest

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