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Corticotropin-releasing factor mediated muscle atonia in pons and medulla

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The dorsolateral pontine inhibitory area (PIA) and medial medullary reticular formation (MMRF) have been found to mediate the muscle atonia of REM sleep. Our previous studies have shown that acetylcholine (ACh) microinjection in the PIA and in the nucleus paramedianus of the medial medulla produces muscle atonia. Glutamate microinjection in both PIA and nucleus magnocellularis (NMC) of the medial medulla also produces muscle atonia. Since immunohistochemical studies have identified corticotropin-releasing factor (CRF) as a potential dorsolateral pontine and NMC transmitter, the present study was undertaken to determine whether this transmitter could produce suppression of muscle tone. Experiments were performed on unanesthetized, decerebrated cats. CRF was microinjected into points in the PIA and NMC at which electrical stimulation produced bilateral inhibition of muscle tone. We found that CRF produced a dose-dependent muscle tone suppression. At 10 nM concentration, the latency and duration of muscle inhibition produced by CRF injection were comparable with those of L-glutamate, at 18.8 s and 4.1 min, respectively. This CRF-induced muscle inhibition was blocked by the CRF antagonist, α-helical [Glu^{27}]corticotropin-releasing factor 9-41 (CRF 9-41). Microinjection of CRF and non-NMDA agonists, kainate and quisqualate, into the same sites in PIA and NMC produced muscle atonia. Pontine sites at which CRF injection induces atonia are identical to those at which acetylcholine microinjection produces atonia. These results indicate that CRF may interact with glutamate and acetylcholine in the generation of muscle atonia.

INTRODUCTION

The phenomenon of muscle atonia in REM sleep was first reported by Jouvet et al.[17]. REM sleep atonia is produced by motoneuron hyperpolarization[5]. The neuronal circuitry involved in this REM sleep atonia includes several regions in the ponto-medullary reticular formation. Carbachol injection into the area ventral to the locus coeruleus corresponding to peri-locus coeruleus alpha (peri-LCα)[35] and adjacent lateral tegmental regions, produces REM sleep-like activity[3,14,43,55]. Electrical or chemical stimulation in this pontine inhibitory area (PIA) and in the medial medulla produces bilateral inhibition of muscle tone in the acute, decerebrate cat[20,22,27].

Electrophysiological and HRP studies have shown that neurons in the PIA project to the medial medulla[36,37,42], which in turn projects to the spinal cord[52]. Lesions of the dorsolateral pons produce the syndrome of REM sleep without atonia[16] as do lesions of the medial medulla[38].

Unit recording studies have localized populations of cells that are selectively active in REM sleep and periods of reduced muscle tone in waking, to the PIA and medial medulla[19,35,41,46]. Medullary REM sleep-on neurons have been shown to be active during the loss of muscle tone seen in cataplectic attacks in narcoleptic dogs[45].

Corticotropin-releasing factor (CRF) has been found in the hypothalamus[2,11] and in extra-hypothalamic regions[8,32,39,50]. Functionally, CRF is not only related to pituitary adrenocorticotropin release[34,53], but also affects the sympathetic nervous system[12,13] and behavior[24,25,29,31,49,51]. However, it is uncertain if CRF plays a role in the sleep–waking cycle or in muscle tone control. Since CRF neurons and fibers have been found in the PIA[32] and project to the atonia related nucleus magnocellularis (NMC) of the medulla[26], the present study was designed to investigate the role of CRF in these regions in the control of muscle tone.

MATERIALS AND METHODS

Experiments were performed on 26 adult cats of either sex. Cats were decerebrated at the precollicular-postmammillary level. Tracheostomy, ligation of carotid arteries, cannulation of both right femoral artery and vein, and decerebration were done under halothane–oxygen anesthesia. Halothane anesthesia was discontinued after decerebration. Neck, triceps brachii, and gastrocnemius

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muscles in the left leg were implanted with bipolar electrodes for electromyographic (EMG) recording. Eye movement was recorded with a pair of screw electrodes placed in the caudal orbit. Blood pressure was recorded with a Statham pressure transducer through polyethylene tubing placed in the femoral artery. Rectal temperature was maintained at 38 ± 1°C through a thermostatically regulated heating pad.

The inhibitory sites in both pons and medulla were identified by electrical stimulation through a stainless-steel monopolar microelectrode (A & M systems), with 500 ms trains of 0.2 ms, 20–100 μA rectangular cathodal pulses at 100 Hz, as previously described[20]. Once the area was identified, 0.5 μl of CRF solution, whose concentration ranged from 0.01 nM to 10 nM, was microinjected through a 1-μl Hamilton (25 sG) microsyringe over a period of 60 s. Injections were also made in some lateral medulla sites according to stereotaxic parameters without prior electrical stimulation. In antagonist studies, α-helical [Glu^{27}]corticotropin-releasing factor 9-41 (α-helical CRF 9-41) was injected 5 min prior to CRF injection at the same site. EMG activity, integrated EMG, and blood pressure were recorded on a Grass Model 78D polygraph. EMG activity change was defined as a change of >30% in integrated EMG magnitude within 1 min of the end of microinjection. Iron was deposited at the injection sites through a stainless-steel monopolar microelectrode at the end of experiments. Brain tissues were sectioned at 60 μ, stained with Neutral red and counterstained with ferrocyanide to identify iron deposits. Stimulation sites were reconstructed according to Berman[4].

CRF was dissolved in either Ringer saline or phosphate buffer, pH 7.2 (Sigma). α-Helical CRF 9-41 (500 nM) was dissolved exclusively in phosphate buffer solution. Kainic acid (KA, 0.2 mM), quisqualic acid (QA, 10 mM), L-glutamic acid diethyl ester (GDEE, 0.2 M), and γ-D-glutamylglycine (DGG, 10 mM) were dissolved in Ringer saline.

RESULTS

Electrical stimulation in pons and medial medulla including the PIA, the region medial to the cuneiformis nucleus (CNF), the dorsal nucleus of the lateral lemnis-

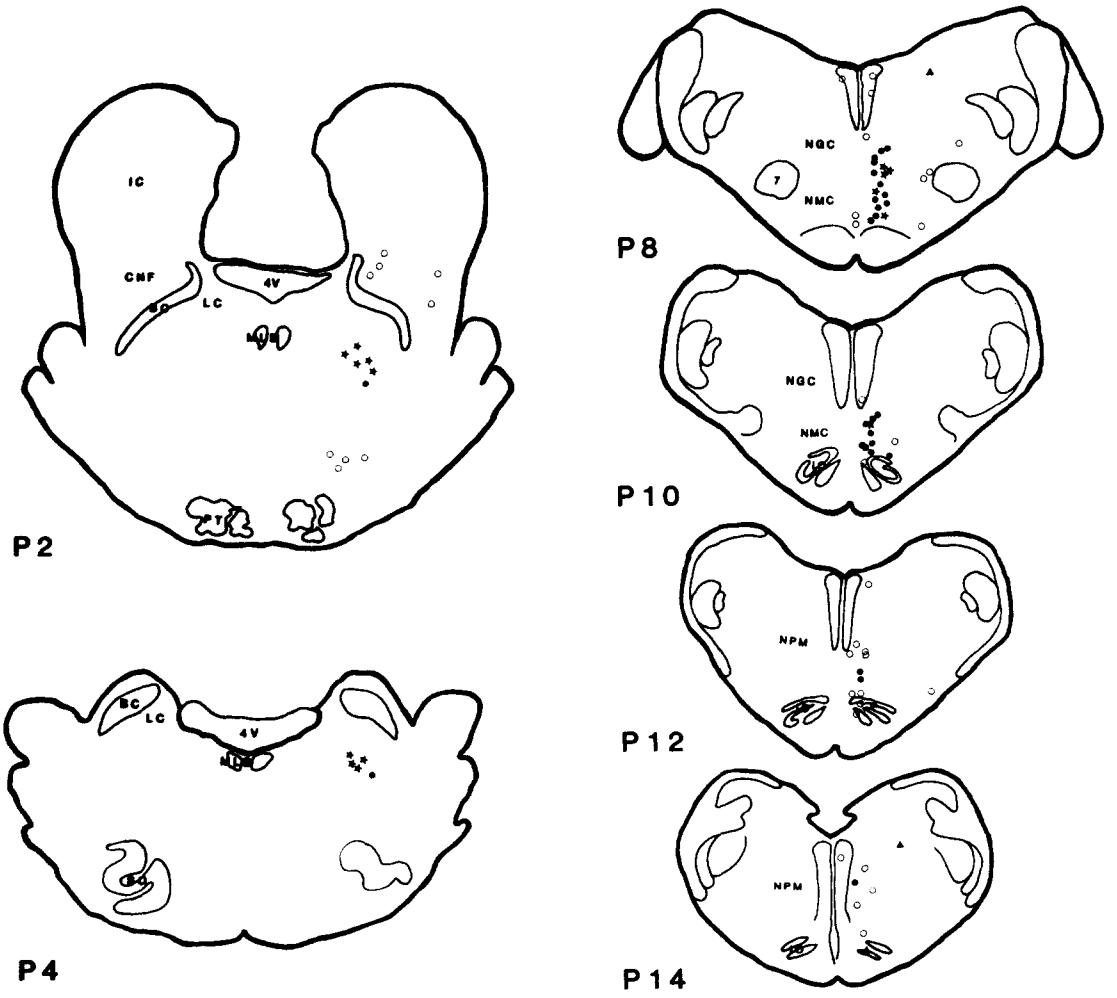

Fig. 1. Location of corticotropin-releasing factor (CRF) injections in pons and medulla. Data is summarized from 26 decerebrated cats. Injections were made in both sides of brainstem. Electrical stimulation produced bilateral inhibition of the muscle tone at all tested sites in pons and medial medulla. The sites in lateral medulla were chosen according to stereotaxic parameters without electrical stimulation. (□), decrease; (▲), increase; (○), no change in muscle tone after CRF injection. (★), decrease of muscle tone after both CRF and non-NMDA agonists injection. 4V, fourth ventricle; 7, facial nucleus; BC, brachium conjunctivum; CNF, nucleus cuneiformis; IC, inferior colliculus; IO, inferior olive; LC, nucleus locus coeruleus; MLB, medial longitudinal bundle; NGC, nucleus gigantocellularis; NMC, nucleus magnocellularis; NPM, nucleus paramedianus; PT, pyramid tract; SO, superior olive.

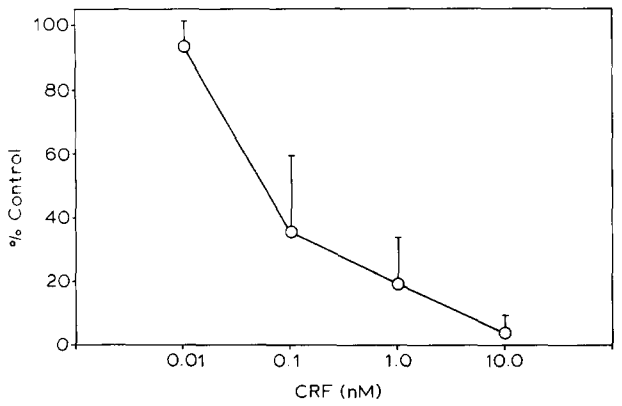

Fig. 2. Dose-dependent effect of CRF on muscle tone. Magnitude was calculated with reference to integrated EMG amplitude in 2 min baseline period. Concentration of CRF was varied from 0.01 nM to 10 nM in counterbalanced order. Injection value was 0.5 μl. Each point is based on the mean activity in the 6 recorded muscles in 6 experiments.

cus, ventral paralemniscal tegmental field (vFTP), nucleus pontis centralis oralis (Poo), ventral part of nucleus gigantocellularis (NGC), NMC, and nucleus paramedianus (NPM) produced bilateral inhibition of muscle tone in the neck and limb muscles. The latency and duration of this inhibitory effect varied as a function of area, as previously reported[21,22]. Eye movements were elicited during stimulation in the ventral part of NGC.

CRF microinjection, into sites at which electrical stimulation produced motor inhibition in PIA, ventral NGC, and NMC, produced muscle tone suppression bilaterally (Fig. 1). This CRF-induced muscle suppression was dose-dependent (Fig. 2). CRF at 0.01 nM (n = 6) produced a small inhibition, detectable in the integrator output, although only barely visible on the polygraph. 10 nM (n = 25) CRF produced a complete suppression of tone in all recorded muscles. The latency and duration of CRF-induced muscle suppression at 10 nM were 18.8 s and 4.1 min (range from 1.75 to 10 min), respectively. Although the duration of CRF-induced muscle suppression was relatively short, the interval between two injections at the same site had to be more than 6 h for the second injection to produce a suppression of the same magnitude as the first one. Injections given within 6 h of prior CRF injections produced less reduction or no change of EMG activity possibly due to receptor desensitization. The effect of CRF on muscle activity was not due to cardiovascular changes. Blood pressure and heart rate remained at the control level throughout the period of muscle inhibition at all injection sites. Eye movement was not seen after CRF injection in the ventral NGC, where electrical stimulation produced eye movement.

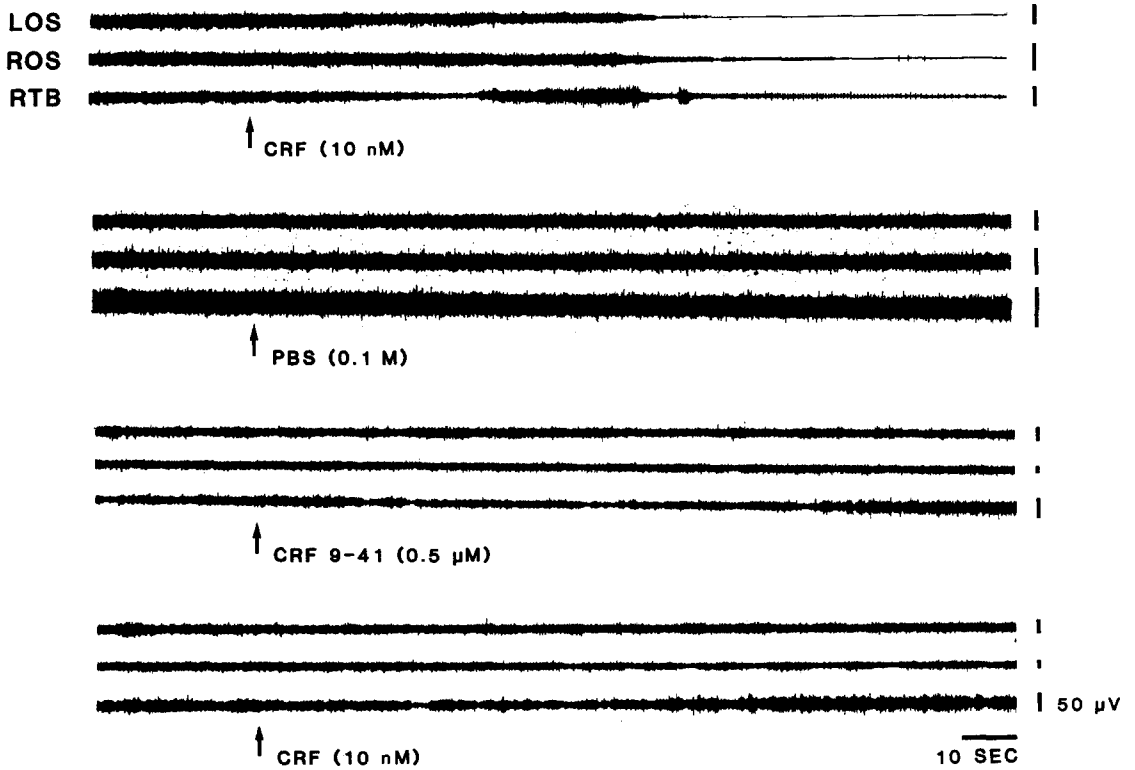

Fig. 3. Effect of CRF on muscle activity. CRF injection in the NMC produced atonia. Control vehicle phosphate buffer solution (PBS) injection did not induce any change of muscle tone 6 h after CRF injection. α-Helical corticotropin-releasing factor 9-41 (CRF 9-41), a CRF antagonist, produced a slight increase in one (RTB) muscle and blocked the effect of CRF on muscle tone which was injected 5 min after it. All injections were made in the same site of NMC. LOS and ROS, left and right occipitoscapularis; RTB, right triceps brachii.

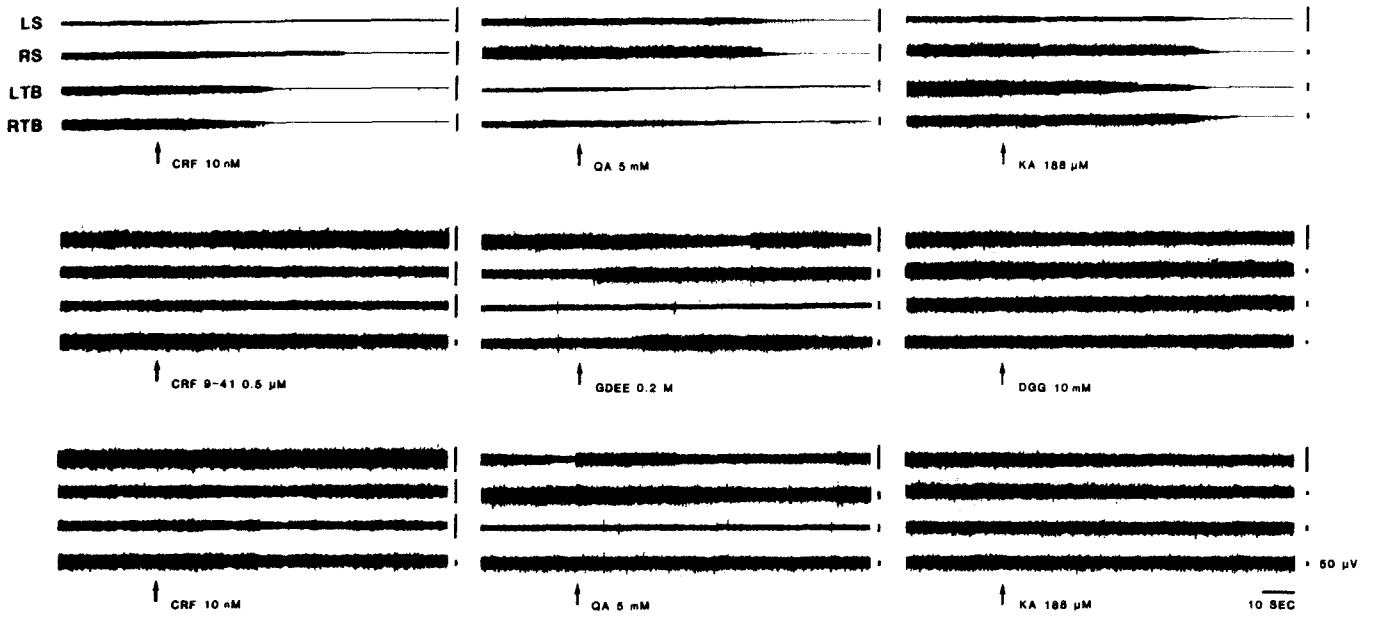

Fig. 4. CRF and non-NMDA agonists quisqualic acid (QA) and kainic acid (KA), all produced muscle atonia (first row). Effects of CRF were blocked by CRF 9-41 and those of QA and KA were blocked by L-glutamic acid diethyl ester (GDEE), and γ-D-glutamylglycine (DGG) (second and third rows), respectively. All chemicals were microinjected into the same site in peri-LCα, a site at which electrical stimulation produced muscle tone suppression. LTB, left triceps brachii.

Defecation was occasionally induced by CRF injection in the pons.

Microinjection of CRF into inhibitory areas identified by electrical stimulation located outside of PIA, vFTP and areas medial and lateral to the CNF produced no change in muscle tone (Fig. 1), although electrical stimulation induced suppression of muscle tone. In inhibitory sites in the nucleus paramedianus of the caudomedial medulla, CRF injection induced no change (10/13) or decreased tone (3/13). Injections in sites in the lateral medulla at which electrical stimulation was not applied, produced no change (5 sites in ventral portion) or increased tone (2 sites in dorsal portion).

Injection of 0.5 μl of the CRF antagonist, α-helical CRF 9-41, 5 min prior to CRF injection, significantly ($P < 0.01$, t-test) attenuated the CRF effect on muscle activity in both PIA and NMC (Fig. 3), with CRF reducing tone to 6 ± 4.2% of baseline levels, while CRF after antagonist injection reduced tone to 92 ± 3.6% of baseline levels. Injection of the CRF antagonist itself produced no significant effect with no change in (6/9 of trials) or slightly increased (3/9 of trials) muscle activity. Control phosphate buffer or Ringer saline injection did not produce any change in EMG activity.

Five cats received both CRF and non-NMDA agonist injections at the same sites in the pons (9 sites) and NMC (6 sites). The result is shown in Fig. 4. Both CRF and non-NMDA agonists including KA and QA[20,23], produced muscle atonia bilaterally. The interval between CRF and prior non-NMDA agonist injections in the same site could be as short as one hour. This was much shorter than the minimum interval between two consecutive CRF injections with undiminished effects on EMG.

DISCUSSION

The present studies demonstrate that CRF application produces atonia in areas that convergent anatomical and physiological data indicate are part of the REM sleep atonia circuit. This CRF-induced muscle atonia could be blocked by CRF antagonist, α-helical CRF 9-41. Furthermore, both CRF and non-NMDA agonist injection into the same sites in PIA and NMC produced muscle atonia. Muscle tone suppression induced by CRF injection was not blood pressure or heart rate related as is the case with glutamate (Glut) and acetylcholine (Ach) atonia induced under the same conditions.

The behavioral response to CRF infusion in chronic, intact animals depends on the site of injection and the environment in which the animals were tested. Intracerebroventricular (ICV) infusion of CRF produces a dose-dependent decrease in locomotor activity compared with control saline infusion in the freely moving rat[29]. In rhesus monkeys, CRF induced lying-down behavior when the animals were in their home cage, while behavioral arousal was found when animals were chair-restrained[18]. Low doses of CRF infused ICV produced dose-dependent EEG desynchronization in the freely moving rat[10].

ICV injection of CRF in the freely moving rabbit produced a decrease in REM sleep[6]. In contrast, CRF in-

jection through the same route in the REM sleep deprived rat increased REM sleep duration[28]. Although CRF alone produced a small but non-significant decrease in REM sleep, CRF infusion restored REM sleep suppressed by interleukin-1 infusion[33]. The extent to which these systemic effects are mediated by the pontine and medullary sites identified in the present work remains to be determined.

ICV infusion of CRF has been found to increase neuronal activity in locus coeruleus and hippocampal pyramidal neurons[1,49,54]. Using the iontophoresis technique and extracellular recording, Eberly et al.[9] found that CRF excited most of the neurons in the cortex and hypothalamus and inhibited neurons in the thalamus and lateral septum. Furthermore, all the neurons in the cortex and diencephalon responding to CRF were also excited by glutamate. We have similarly found both glutamate and CRF effects at the same sites in PIA and NMC in the present study. This suggests that these agonists are either acting on neurons with both CRF and Glut receptors, or on co-localized groups of cells having these receptors.

CRF has been found to co-exist with other neurotransmitters in the central nervous system. Coexistence of CRF- and neurotensin-like immunoreactive neurons has been found in lateral bed nucleus of the stria terminalis and central amygdaloid nucleus[30,40] which projects to the dorsolateral pons. Extensive co-localization of CRF and Met-enkephalin immunoreactivity has been reported in the hypothalamus[15]. In the dorsolateral tegmental nucleus of the brainstem, and in the pedunculopontine nucleus, CRF was found to co-exist with substance P and Ach[7,48]. Cholinergic mechanisms in PIA participate in REM sleep triggering[44]. Although there is not yet evidence for a co-projection of CRF and acetylcholine to PIA, we hypothesize that CRF release, along with ACh and glutamate in PIA and with glutamate in NMC, plays a role as a transmitter in the control of REM sleep atonia.

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