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Rostral Ventral Medulla Cholinergic Mechanism in Pain-Induced Analgesia

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Abstract

The ascending nociceptive control (ANC), a novel spinostratial pain modulation pathway, mediates a form of pain-induced analgesia referred to as noxious stimulus-induced antinociception (NSIA). ANC includes specific spinal cord mechanisms as well as circuitry in nucleus accumbens, a major component of the ventral striatum. Here, using the trigeminal jaw-opening reflex (JOR) in the rat as a nociceptive assay, we show that microinjection of the nicotinic acetylcholine receptor (nAChR) antagonist mecamylamine into the rostral ventral medulla (RVM) blocks NSIA, implicating RVM as a potentially important link between ANC and the PAG – RVM – spinal descending pain modulation system. A circuit connecting nucleus accumbens to the RVM is proposed as a novel striato-RVM pathway.

Keywords

nucleus accumbens; noxious stimulation; antinociception; pedunculo pontine tegmental nucleus; acetylcholine

Introduction

Pain can be modulated through activation of neural pathways by various physiologically relevant environmental stimuli. For example, noxious (i.e., painful) events can trigger the induction of pain-induced analgesia [8,10]. One form of pain-induced analgesia, noxious stimulus-induced antinociception (NSIA), is mediated by a novel spinostratial pathway termed ascending nociceptive control (ANC) [10].

ANC can induce analgesia comparable to that of high dose morphine when activated by painful stimuli such as subdermal capsaicin administration [10]. This analgesic effect can be detected remotely from the site of stimulation: capsaicin administration to the hind paw of the rat can be observed as attenuation of the trigeminal jaw-opening reflex (JOR) [10,24]. Previous studies have implicated specific ANC circuits in the spinal cord [28–30] as well the ventral striatum [10,22–24] that mediate NSIA; however, the mechanisms downstream of the ventral striatum by which ANC modulates nociception remain to be discovered. Although the opioidergic PAG–RVM– spinal cord pain modulation system [1,9] is a likely candidate for this circuit,

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naloxone injected into either PAG or RVM does not block ANC-mediated analgesia [11]. In the present study we examined the involvement of acetylcholine nicotinic receptors (nAChR) in the RVM; previous studies have implicated these receptors in nociceptive modulation [4, 7,14,15,19].

Materials and methods

Experiments were performed on 280–350 g male Sprague-Dawley rats (Charles River, Hollister, CA). Experiments were approved by the Institutional Animal Care and Use Committee at UCSF and adhered to the guidelines of the American Association of Laboratory Animal Care, National Institutes of Health, and the Committee for Research and Ethical Issues of the International Association for the Study of Pain. Effort was made to minimize the number of animals used and their suffering.

Experiments were performed in rats anesthetized with an intraperitoneal injection of 0.9 g/kg urethane and 45 mg/kg α -chloralose (both from Sigma-Aldrich, St. Louis, MO). This method provides a state of anesthesia with stable physiological parameters [5] and a stable jaw-opening reflex (JOR) electromyographic (EMG) signal [11] over the time period required to complete the experiments.

Changes in nociception were measured as attenuation (i.e., antinociception) or enhancement (i.e., hyperalgesia) of the JOR electromyographic (EMG) signal [10,11,18]. This assay was employed because it is segmentally remote from the hind paw where the noxious stimulus is applied, thus allowing separation of heterosegmental effects from any intrasegmental effects that might influence assays like the paw-withdrawal reflex or the tail flick reflex. Previous studies show examples of the effect of intraplantar capsaicin on the JOR EMG traces [24].

To evoke the JOR, a bipolar stimulating electrode, consisting of two insulated copper wires (36 AWG), each with 0.2 mm of insulation removed from the tips, one tip extending 2 mm beyond the other, was inserted into the pulp of a mandibular incisor to a depth of 22 mm from the incisal edge of the tooth to the tip of the longest wire and cemented into place with dental acrylic resin. A bipolar recording electrode, consisting of two wires of the same material as the stimulating electrode with 4 mm of insulation removed, was inserted into the anterior belly of the digastric muscle ipsilateral to the implanted tooth to a depth sufficient to completely submerge the uninsulated end of the wire.

Dental pulp stimulation current was set at 3 times EMG threshold. Stimuli consisted of 0.2 ms square wave pulses at a frequency of 0.33 Hz. Each data point consisted of the average peak-to-peak amplitude of 12 consecutive JOR EMG signals. Baseline JOR amplitude was defined as the average of 3 data recordings prior to an intervention. Data were normalized for differences in baseline by calculating the percentage change from baseline for each post-intervention data point. These values were used in the statistical analyses and were also plotted in the figure.

For intra-RVM drug administration, bilateral 23 gauge stainless steel guide cannulae were stereotaxically positioned (2.3 mm caudal and 0.5 mm ventral to the intra-aural line, and 1 mm either side of the midline) and fixed into place with orthodontic resin (L.D. Caulk Co., Milford, DE, USA). Drug administration was accomplished via insertion of a 30 gauge stainless steel injection cannula, which extended 2 mm beyond the guide cannula, connected to a 2 μ l syringe (Hamilton, Reno, NV, USA). Injection volumes were 0.5 μ l and were carried out over a period of 2 minutes after which the cannula was left in place an additional 30 seconds. Administration sites were verified by histological examination and were plotted on coronal maps adapted from the atlas of Paxinos and Watson [20].

The nAChR antagonist mecamylamine (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in phosphate buffered saline (PBS). Capsaicin (E-capsaicin, Sigma-Aldrich) was initially dissolved in Tween 80 (50%) and ethanol (50%) to a concentration of 50 µg/µl and then diluted with 0.9% saline to a concentration of 5 µg/µl; subdermal capsaicin injection volume was 50 µl (250 µg) in all experiments.

A two-way repeated measures ANOVA with one between-subjects factor (i.e., treatment) and one within subjects factor (i.e., time) was used to determine if there were significant ($p \leq 0.05$) differences in nociceptive responses among the groups.

Results

To determine if nAChRs in the RVM mediate NSIA, mecamylamine (0.6 µg) or its vehicle, PBS, was bilaterally administered to RVM 10 minutes prior to capsaicin administration in the plantar surface of a hind paw. NSIA was markedly diminished in the group receiving mecamylamine compared to the group receiving PBS (Fig. 1A), whereas mecamylamine alone had no significant effect. The main effect of treatment group was significant ($F_{2,31}=36.379$, $p < 0.001$). Scheffé post hocs demonstrated that the two groups receiving mecamylamine were significantly different from the group that received capsaicin alone ($p < 0.001$ in both cases), but not different from each other ($p = 0.871$). There was also a significant group $\times$ time interaction ($F_{8,84}=13.180$, $p < 0.001$) and a significant main effect of time ($F_{4,84}=3.958$, $p = 0.005$). RVM injection sites are shown in Figure 1B.

Discussion

This study provides evidence that ANC is mediated by a cholinergic mechanism in the RVM. Previous reports have implicated intra-RVM cholinergic mechanisms in nociceptive modulation. Direct intra-RVM injection of agonists for either nicotinic or muscarinic cholinergic receptors have been shown to produce antinociception [4,7,14,15,19], and cholinergic antagonists for either nicotinic or muscarinic receptors injected in the RVM diminish the antinociceptive effect cholinergic agonists co-injected into the RVM [14].

While a few intrinsic cholinergic neurons have been reported in the RVM [3,16,25], any role they play in ANC would depend on a yet-to-be identified afferent activating mechanism. Although the RVM is well known to receive afferents from the PAG, available evidence argues against PAG participation in RVM cholinergic mechanisms [2]. The most likely source for RVM cholinergic activity relevant to ANC is an afferent projection originating in pedunculopontine tegmental nucleus (PPTg), located in the mesopontine tegmentum [14,21,32]. Electrophysiological evidence supports a role for the PPTg in nociceptive processing [6], and pharmacological activation of these neurons in the PPTg produces antinociception that can be blocked by cholinergic antagonists microinjected into the RVM [14].

Although speculative, it is possible to propose a circuit connecting nucleus accumbens to the RVM via the PPTg. The ventral striatum, of which nucleus accumbens is a major component, is characterized by two output systems, the striatopallidal (“indirect”) pathway and the striatonigral (“direct”) pathway (reviewed by Gerfen, 1992 [12]). Virtually all ventral striatal output neurons are GABAergic medium spiny neurons; however, striatopallidal and striatonigral neurons can be distinguished on the basis neurotransmitter content, peptide content, and dopamine receptor type [12,17]. One of these distinguishing features is that striatopallidal neurons express dopamine D₂-receptors, while striatonigral neurons express D₁-receptors [13]. Since, in an earlier study, we found that ANC is mediated by intra-accumbens D₁- but not D₂-receptors [22], it seems reasonable to speculate that the striatonigral pathway is a component of ANC. Moreover, one of the two specific targets of the striatonigral

pathway is the substantia nigra pars reticulata (SN_{PR}) [12], which projects to the PPTg [26]. Thus, we propose that the ANC circuit from nucleus accumbens to the RVM is through the striatonigral pathway, to the SN_{PR}, the PPTg, and then to the RVM.

The RVM is now accepted as important in pain facilitation as well as pain inhibition [31], and we have shown previously that ventral striatal mechanisms can increase pain [23]. In nicotine-tolerant rats, intra-accumbens mecamylamine microinjection induced significant hyperalgesia. Thus, given our current findings demonstrating RVM nAChR involvement in ANC, it is not unreasonable to propose the existence of a striato-RVM hyperalgesia circuit, although the details of such a mechanism have yet to be worked out.

Activation of nAChRs in the spinal cord has been shown to enhance inhibitory post-synaptic potentials (IPSPs) [27], a mechanism that could possibly explain the effect of nAChR activation on NSIA in the RVM as well as in nucleus accumbens [23].

In summary, we have shown that ANC is mediated by a cholinergic mechanism in the RVM, thereby adding a significant new dimension to this novel spino-striato-RVM pain modulation circuit. Although it is possible that this new mechanism represents a hitherto undiscovered element of the PAG-RVM pain modulation system, that system is mediated by endogenous opioids in both PAG and RVM, whereas ANC is not. Therefore, any functional connection between the two systems remains to be established.

Acknowledgments

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B

Figure 1. Effect of intra-RVM mecamylamine on NSIA

A. Intra-RVM mecamylamine blocked NSIA but had no significant effect itself. Data are presented as percentage attenuation of the JOR EMG (mean $\pm$ s.e.m.). Numbers in parentheses designate the number of rats in the groups. **B.** RVM injection sites plotted on drawings adapted from the atlas of [20]. Numbers represent distance caudal of the intra-aural line. Some symbols overlap others. Closed circles, group that received mecamylamine prior to capsaicin injection; open circles, group that received mecamylamine alone.