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Contribution of the Periaqueductal Gray to the Suppression of Pain Affect Produced by Administration of Morphine Into the Intralaminar Thalamus of Rat

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Abstract: The parafascicular nucleus (nPf) of the intralaminar thalamus is implicated in the processing of pain affect in both animals and humans. Administration of morphine into nPf results in preferential suppression of the affective reaction to noxious tail shock in rats. The involvement of the ventrolateral periaqueductal gray in mediating the antinociceptive action of morphine injected into nPf was evaluated. Vocalizations that occur after tail shock offset (vocalization afterdischarges) are a validated rodent model of pain affect and were preferentially suppressed by injection of morphine into nPf. Vocalizations that occur during tail shock were suppressed to a lesser degree, whereas spinal motor reflexes (tail flick and hind limb movements) were unaffected by injection of morphine into nPf. Inactivation of the vPAG via the microinjection of muscimol ($GABA_A$ agonist) produced dose-dependent antagonism of morphine-induced increases in vocalization thresholds. The results demonstrate that a functional link between the nPf and vPAG in generating the antinociceptive action of morphine injected into nPf.

***Perspective:** Microinjection of morphine into nucleus parafascicular preferentially suppressed rats' affective reaction to noxious stimulation. This affective analgesia was reversed by inactivation of the ventrolateral periaqueductal gray. Understanding the neurobiology underlying the suppression of pain affect will provide insights into new treatments for pain and its associated affective disorders.*

***Key words:** Nucleus parafascicularis, ventrolateral periaqueductal gray, morphine, pain affect, vocalizations.*

The affective dimension of pain motivates those in pain to seek medical care and contributes to the development of emotional disturbances such as anxiety, fear, and depression that contribute to the suffering of patients in pain.[35] Successful pain management therefore requires therapeutic strategies directed toward alleviating its affective attributes. The development of these strategies necessitates an understanding of the neurobiology that processes and modulates pain affect.

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The intralaminar thalamic parafascicular nucleus (nPf) is implicated in the processing of pain affect. It receives nociceptive afferents[22,33] and noxious peripheral stimulation evokes neural activity in nPf.[17,60] Ablation of nPf relieves the emotional suffering associated with chronic pain in humans[40,61] and reduces affective responses of animals to noxious stimulation.[18,30,53] Reduced pain reports of patients with Alzheimer's are correlated with neuronal degeneration in nPf.[52,55] Alternately, high frequency stimulation of nPf results in reports of intense pain and unpleasantness in humans[58,59] and aversive pain-like reactions in animals.[29,51]

μ-Opioid receptors are localized within the nPf in rats and humans[21,37] and the iontophoretic application of morphine into nPf of rats inhibited neural activity elicited by noxious stimulation of the tail.[49] The significance of these receptors is indicated by reports that systemic administration of morphine suppressed noxious-evoked neural activity elicited in nPf of cats.[32]

Additionally, the intense pain accompanying nPf stimulation in humans was suppressed by the systemic administration of μ-opioid agonists.[59]

We reported that microinjection of morphine into nPf preferentially suppressed rats' affective reaction to noxious tail shock.[27] The present study evaluated the contribution of the ventrolateral periaqueductal gray (vPAG) to this antinociceptive action. The vPAG is a core midbrain site that underlies antinociception.[1,6] Antinociception elicited from vPAG is mediated by descending projections that engage inhibitory spinopetal projections of the rostral ventromedial medulla (RVM) that suppress nociceptive transmission within the spinal dorsal horn. Ascending projections from the vPAG to the limbic forebrain and thalamus also contribute to the antinociception elicited by vPAG-stimulation or morphine injections into the vPAG. Conversely, antinociception elicited from the limbic forebrain (anterior cingulate cortex, amygdala, habenula) is mediated through projections to the vPAG.[34,46]

A functional interaction between vPAG and nPf is indicated by findings that suppression of pain affect in rats produced by morphine administered into vPAG was blocked by injecting methysergide (serotonin antagonist) into nPf.[6] Morphine injected into vPAG also suppressed noxious-evoked neural activity in nPf that was reversed by iontophoretic application of methysergide into nPf.[17] A reciprocal interaction between nPf and vPAG is indicated by reports that stimulation of nPf activates the vPAG,[54] injection of morphine into nPf activates a subset of non-nociceptive responsive neurons in the nPf,[16] and vPAG receives direct projections from nPf.[38]

For the present study, the antinociceptive action of morphine injected into nPf was assessed after inactivation of the vPAG by microinjection of the $GABA_A$ agonist muscimol.[41] Based on our earlier report, we predicted that intra-nPf administered morphine would preferentially suppress rats' affective reaction to noxious tail shock. Previous research in this laboratory validated vocalization afterdischarges (VADs) as a rodent model of pain affect (see Discussion). VADs occur immediately after application of noxious tail shock, are organized within the forebrain, and have distinct spectrographic characteristics compared with vocalizations that occur during shock (VDSs). We predicted that morphine injected into the nPf would preferentially elevate VAD threshold compared with thresholds of tail shock-elicited behaviors organized at spinal (SMR = hind limb movements and tail flexion) and medullary (VDS) levels of the neuraxis.[8,15] If the antinociceptive action of morphine injected into nPf is mediated via the vPAG, then it will be attenuated by administration of muscimol into vPAG.

Materials and Methods

Animals

Male Long-Evans rats (Charles River, Raleigh, NC) ranging from 90 to 150 days old were used. Rats were housed as pairs in plastic cages in a climate controlled vivarium (lights on 6 AM to 6 PM), and given ad libitum access to food and water. Testing occurred during the light portion of the cycle. Rats were handled 1 to 2 times per day for at least 1 week before testing to minimize possible effects of stress from human contact. All procedures were approved by the Animal Investigation Committee of Wayne State University and followed international guidelines.

Surgery and Histology

Surgeries were performed under aseptic conditions. Rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.) after pretreatment with atropine sulfate (1 mg/kg i.p.), and positioned in a Kopf small animal stereotaxic frame. All implants were made with single or double stainless steel 22-gauge cannulae (Plastics One, Roanoke, VA) after stereotaxic coordinates adapted from the rat brain atlas of Paxinos and Watson[47] and measured relative to the bregma suture and the top of the level skull. Guides were implanted unilaterally at a 10° angle (lateral to medial) 2 mm above the vPAG. During the same surgical session bilateral guide cannulae were implanted 2 mm directly above the nPf. The following coordinates (in millimeters) were used: nPf (bilateral, AP = −4.3, L = ± 1.2, DV = −4.0) and vPAG (unilateral, AP = −7.5, L = ± 0.6, DV = −3.8). Guides were affixed to the skull with 4 stainless steel bone screws and cranioplastic cement. Each guide cannula was fitted with a 28-gauge dummy cannula that extended the length of the guide to keep it clear of debris. Rats were given 7 to 10 days to recover before initiation of testing.

After testing, rats were killed by carbon dioxide asphyxiation. Injection sites were marked by safrin-O dye and brains were extracted and placed in a 20% (wt/vol) sucrose formalin solution for 48 to 72 hours. Brains were sectioned at 50 μm on a freezing microtome, and injection sites were localized with the aid of the Paxinos and Watson[47] brain atlas by an experimenter unaware of the behavioral outcomes.

Apparatus

Testing was controlled by custom computer programs via a multifunction interface board (DT-2801, Data Translation, Marlboro, MA) installed in a PC. Rats were placed into custom made Velcro body suits and restrained on a Plexiglas pedestal using Velcro strapping that passed through loops located on the underside of the suits (see photograph in Reference 5). This design maintained rats in a crouching posture throughout testing, enabled them to breathe and vocalize normally, and permitted unobstructed access to the head for intracerebral injections. Testing was conducted within a sound attenuating, lighted, and ventilated chamber equipped with a small window that enabled visual monitoring of rats during testing.

Tail shock (20-ms pulses at 25 Hz for 1,000 ms) was delivered by a computer controlled constant current shocker (STIMTEK, Arlington, MA) through electrodes (0-gauge stainless steel insect pins) placed intracutaneously