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Opiate and stimulus-produced analgesia: Functional anatomy of a medullospinal pathway

(dorsal horn neurons/nucleus raphe magnus/trigeminal system/morphine)

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ABSTRACT **Neurons in ventromedial medulla, including the nucleus raphe magnus, project to trigeminal nucleus caudalis and, via the dorsolateral funiculus, to spinal dorsal horn. The terminals of this descending system are in loci containing cells responsive to noxious stimuli. Electrical stimulation of nucleus raphe magnus selectively inhibits spinal dorsal horn neurons that respond to noxious stimuli. These neurons are located near the anatomically demonstrated terminals of this descending system. Dorsolateral funiculus lesions block this descending inhibition of spinal neurons as well as the analgesic action of morphine. This evidence supports the hypothesis that this neuron population mediates the analgesia produced by opiates and electrical stimulation of certain diencephalic and brainstem sites.**

Electrical stimulation of discrete medial diencephalic and brainstem sites gives rise to profound analgesia in awake, freely moving animals, including man (1–4). Analgesia can also be produced by microinjection of opiate drugs into many of these same regions (5, 6). Recently, an endogenous pentapeptide, enkephalin, with opiate-like pharmacological actions has been identified (7). The regional distribution of enkephalin (8), as well as that of opiate-binding sites (9), overlaps with the effective regions for stimulus-produced analgesia. Furthermore, naloxone, a specific opiate antagonist, counteracts stimulus-produced analgesia (4, 10). Taken together, these studies support the view that opiate and stimulus-produced analgesia are mediated by a common neural mechanism (2, 3).

Both opiate and stimulus-produced analgesia appear to depend on descending connections to spinal cord (3, 11). Periaqueductal gray stimulation produces analgesia, inhibition of withdrawal reflexes (2), and preferential inhibition of dorsal horn neurons sensitive to noxious stimulation (12). At present, there is no evidence for a direct periaqueductal gray–spinal cord projection, but the periaqueductal gray does project to the nucleus raphe magnus (NRM), a large celled, serotonin-containing component of the midline raphe nuclear groups located in the medulla just dorsal to the pyramids (13, 14). Since NRM stimulation also gives rise to powerful analgesia (15) and since NRM appears to contain neurons projecting to the spinal cord (16, 17), the possibility arises that the analgesia and the inhibition of spinal pain transmission cells by opiates and by periaqueductal gray stimulation are mediated via NRM. Our report describes anatomical, physiological, and behavioral evidence for a descending inhibitory action of NRM on spinal and trigeminal neurons that respond to noxious stimulation.

METHODS

For anatomical studies, small quantities (0.1–0.5 μl) of tritiated amino acid (L-[4,5^{3}H]leucine, specific activity 30–55 Ci/mmol) were injected into the NRM of cats. The leucine was desiccated under vacuum and reconstituted with sterile, normal saline to a final concentration of 80–100 μCi/μl. Injections were made stereotaxically with a 10 μl Hamilton syringe, using a 30-gauge fixed needle. The syringe was fixed to a Kopf hydraulic microdrive which pulsed the solution at 1 μm/s, so that total injection times ranged from 45 min to 1 hr. Injections into NRM exclusively are difficult; some spread into adjacent reticular formation is unavoidable. For this reason, control injections were made into the nucleus gigantocellularis (a region that produces pain upon electrical stimulation).

Survival times of 24 hours or 11 days were used in order to study fast and slow components of axoplasmic transport. The cats were perfused under deep barbiturate anesthesia with a 4% (wt/vol) paraformaldehyde, ½% (wt/vol) glutaraldehyde, phosphate-buffered fixative. Thirty micrometer frozen sections through the brain and spinal cord were cut, mounted on slides, dipped in Kodak NTB-2 emulsion (diluted 1:1 with water), dried, and placed in light-tight boxes at 4° for periods of 1–3 months. At the appropriate times, the slides were developed in D-19, washed, and fixed in Kodak Rapid Fix, without hardener. Alternate sections were stained with neutral red and examined with both light- and dark-field illumination.

Physiological experiments were carried out to examine the effects of NRM stimulation on the response of spinal cord dorsal horn neurons to natural stimulation. Extracellular recordings of lumbosacral dorsal horn neurons were made in cats that were electrolytically decerebrated at the midcollicular level under halothane anesthesia (18). After decerebration, anesthesia was discontinued. Concentric bipolar stimulating electrodes (0.5 mm in diameter, 0.3 mm tip separation) were stereotaxically placed in NRM. The activity of dorsal horn neurons was monitored with 4–10 MΩ, 0.5 M K^+ acetate-dye filled glass micropipettes. Neurons were tested before, during, and after brief stimulation trains to NRM (100 ms duration, 10 pulses per train, 200 μs pulse width). Current intensities less than 500 μA were used; threshold for inhibition was in the 100- to 300-μA range. Dorsal horn cells were tested for their response to graded natural stimuli. Innocuous stimuli included brushing of hairs and skin and joint manipulation. Noxious stimuli were produced by pinching skin with fingers or toothed forceps. Cells were located by iontophoretic injection of pontamine blue or by their position in a penetration relative to marked neurons.

In a third set of experiments, behavioral observations were made on rats with various spinal cord lesions, using morphine to induce analgesia. The rat was chosen for this study because, unlike in the cat (19), most restricted lesions of the spinal cord do not disrupt the rat's response to a noxious stimulus (20). Furthermore, the location of the corticospinal tract, in the base of the dorsal columns, makes it possible to cut the dorsolateral funiculus without producing hyperreflexia or paralysis. Under barbiturate anesthesia, a midthoracic laminectomy was carried out, and lesions were placed in various parts of the spinal cord of 14 rats. Two sham-operated controls were also prepared.

Abbreviation: NRM, nucleus raphe magnus.

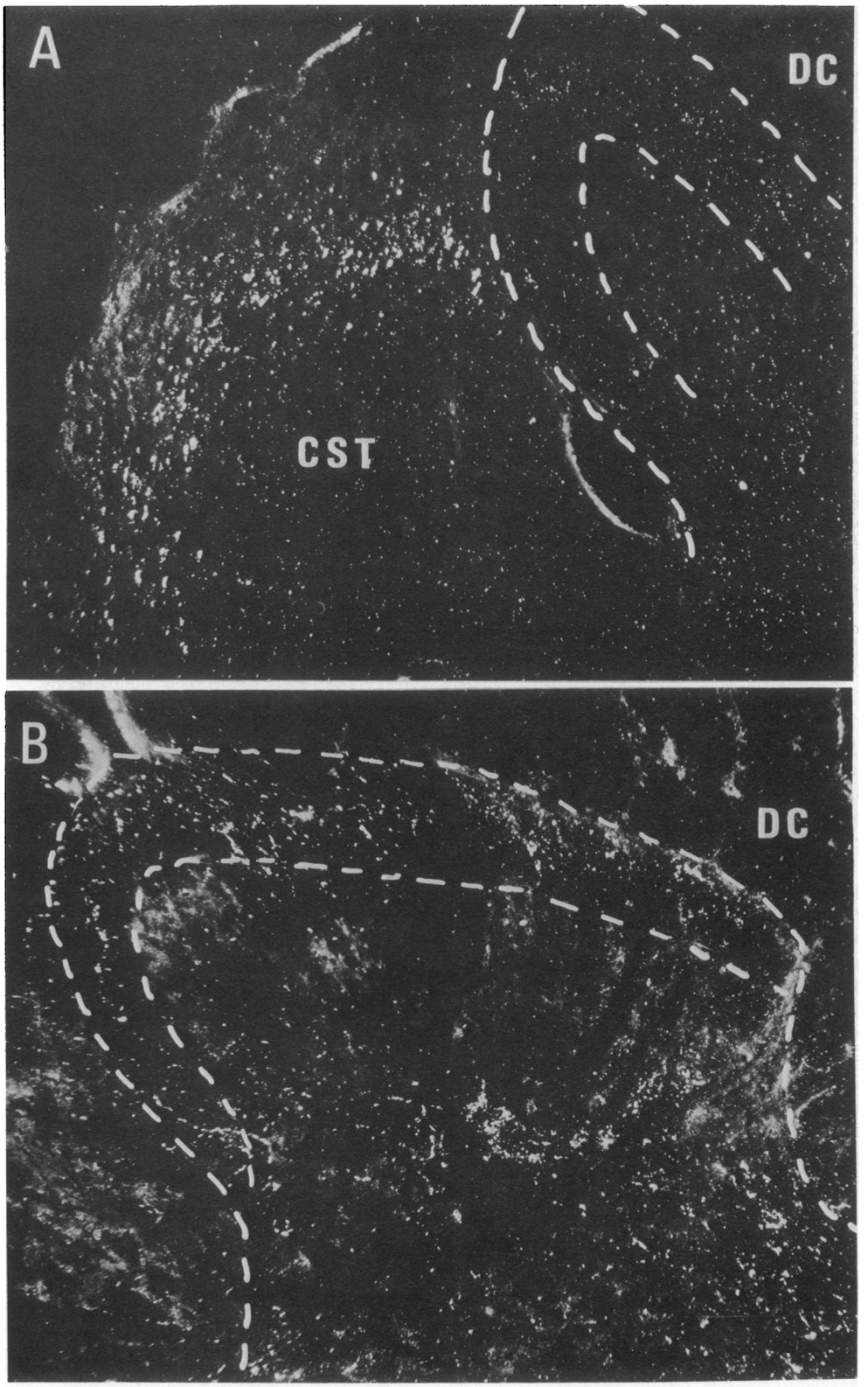

FIG. 1. Spinal cord projections of nucleus raphe magnus. Autoradiograms of cervical cord. Interrupted white lines define the dorsal and lateral boundaries of the dorsal horn and the ventral border of the substantia gelatinosa. (A) C1 level; animal sacrificed 11 days after injection of tritiated leucine. The descending fibers form a dense crescent in the dorsolateral funiculus, lateral to the corticospinal tract (CST). DC, dorsal columns. (B) C8 level; same animal, demonstrating terminal fields over laminae I, II, V, and medial VI.

After recovery from the acute effects of surgery, behavioral observations were made. The observers did not know which animals had spinal cord lesions. On alternate days, the response of rats to application of a toothed alligator clip to the limbs was observed. Behavioral criteria of pain, including vocalization, orientation, and attempts to remove the clip were used. In

normal rats, these behavioral responses to the toothed alligator clip are completely suppressed within 15 minutes after morphine administration (5 mg/kg intraperitoneally). At this dose, rats are still awake and responsive to innocuous environmental stimuli. After behavioral testing was completed, animals were sacrificed and their spinal cords were removed for histological study.

RESULTS

After injections of tritiated leucine into NRM and adjacent ventromedial reticular formation, autoradiographic material revealed a large descending projection to spinal and medullary sites containing neurons known to respond to noxious stimuli. In the medulla, a major terminal field is found in the dorsalmost part (marginal and gelatinosa layers) of the nucleus caudalis of the spinal trigeminal complex, throughout its rostral-caudal extent. A second dense terminal distribution is found at the ventromedial border of the magnocellular layer, including the subadjacent reticular formation. Cells in both these regions contain neurons which respond to noxious stimulation (21). NRM does not distribute to the magnocellular layer of nucleus caudalis, which contains neurons primarily responsive to innocuous stimuli (21).

The major projection to the spinal cord descends in the dorsal part of the lateral funiculus (Fig. 1A), confirming previous retrograde degeneration and fluorescent histochemical studies (16, 17). Fibers terminate in the dorsal horn of all spinal levels. On the basis of studies with fast axoplasmic flow, it appears that the density of the projection is greatest at cervical segments.

As in the nucleus caudalis, a discrete projection is found over the marginal zone and substantia gelatinosa of the dorsal horn [laminae I and II of Rexed (22); Fig. 1B]. A second, dense terminal field is found in lamina V and medial VI. Laminae I and V contain most of the dorsal horn neurons that respond maximally to noxious stimulation (23, 24). In contrast, most neurons in lamina IV, the functional analog of the magnocellular division of nucleus caudalis, respond maximally to innocuous stimuli (24). Lamina IV receives a much smaller projection from NRM than lamina I, II, or V.

Injections into adjacent gigantocellular reticular formation produce a completely different projection pattern. The heaviest distribution is to cranial motor nuclei, the medial accessory olive and, consistent with previous studies (25), the ventral horn of the spinal cord by way of the ventral and ventrolateral funiculi. No projection from gigantocellular reticular formation to nucleus caudalis or to the dorsal horn was found. Thus, the nucleus gigantocellularis projects to motor, rather than sensory, areas of the brainstem and spinal cord.

Physiological studies of the effect of NRM stimulation on spinal dorsal horn neurons were consistent and reflected the anatomical results described above. Of 17 cells that responded with an increasing discharge as stimulus intensities became noxious, 15 were inhibited by NRM during natural stimulation of the cell's receptive field, confirming a preliminary report (26). The inhibition outlasted the stimulation by approximately 200 to 300 ms. The inhibited cells were located histologically in or adjacent to lamina V of the dorsal horn (Fig. 2). Changes in spontaneous activity were difficult to assess because of the low spontaneous activity characteristic of decerebrate preparations. Three of four cells located in lamina I of the dorsal horn were also inhibited. Two of these three responded maximally to noxious stimulation. In contrast, none of the 16 lamina IV-type cells (which respond maximally to innocuous mechanical stimulation) were inhibited. A unilateral lesion of the dorsolateral funiculus at L1 reduced the descending inhibitory effect,

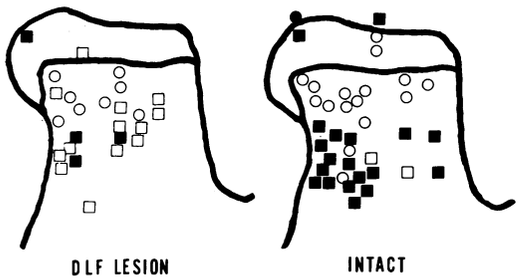

FIG. 2. Diagrams of lumbosacral dorsal horn showing locations of recorded neurons. Circles represent cells responding maximally to innocuous stimuli; squares represent cells responding maximally to noxious stimuli, most of which also respond to lower stimulus intensities. Filled symbols represent cells inhibited by electrical stimulation of nucleus raphe magnus; open symbols are cells unaffected by stimulation. Diagram on left is reconstructed from cells located ipsilateral and caudal to a lesion of the dorsolateral funiculus (DLF) at L1.

so that caudal and ipsilateral to the lesion, inhibition following NRM stimulation was observed in only 4 of 18 units maximally responsive to noxious stimulation. When inhibition was present, it was weaker than on the control side. None of the eight lamina IV-type cells ipsilateral to the lesion were inhibited. Lesions of the ventral part of the lateral funiculus did not lessen inhibition. In one animal, an extensive cord lesion which spared only the dorsolateral funiculus on one side was made. In this preparation, the inhibition of neurons responding to noxious stimulation was preserved ipsilateral to the intact dorsolateral funiculus. All lesions were verified histologically.

Previous studies have demonstrated that analgesia from stimulation of periaqueductal gray sites in the rat is totally abolished in the ipsilateral hindlimb caudal to a dorsolateral funiculus lesion (27). This abolition of stimulus-produced analgesia may be due to interruption of descending inhibitory connections from NRM. Since opiates and stimulus-produced analgesia appear to employ a common neural mechanism (2, 3), we extended these observations using morphine as the analgesic agent.

In all rats with midthoracic lesions of the dorsolateral funiculus, 5 mg/kg intraperitoneal morphine was observed to produce analgesia of the forelimbs, but had little analgesic effect upon responses produced by a toothed alligator clip placed on parts of the ipsilateral body caudal to the lesion. When the clip was applied to a hind limb, the rats vocalized, made vigorous attempts to remove the clip and showed clear signs of agitation. Bilateral lesions of the dorsolateral funiculus were more effective than unilateral lesions. Sham-operated rats and those with lesions sparing the dorsolateral funiculus were not different from normal rats. At higher doses of morphine (20–25 mg/kg), pain responses were abolished caudal to the lesions, and no differences between forelimb and hindlimb were detectable. The reversal of morphine analgesia by bilateral dorsolateral funiculus lesions has recently been confirmed using a standard tail-flick test (33).

DISCUSSION

These results demonstrate a direct projection to spinal cord from regions in ventromedial medulla, including nucleus raphe magnus and adjacent magnocellular reticular formation. This projection is to loci of spinal cord dorsal horn neurons with high intensity inputs and is via the dorsolateral funiculus. The physiological studies indicate that nucleus raphe magnus stimulation exerts an inhibitory effect specifically on neurons

receiving high threshold inputs. This is consistent with the evidence that NRM stimulation produces powerful analgesia in cats (15) and suggests that the analgesic action is at least partially mediated by axons of NRM-neurons. This same pathway may also mediate reticular formation control of flexor reflex afferent actions (28).

Taken in conjunction with the behavioral observations on rats with morphine-induced analgesia, the physiological and anatomical data indicate that lesions of the dorsolateral funiculus block morphine and stimulus-produced analgesia by disrupting a descending inhibitory pathway from nucleus raphe magnus to the spinal dorsal horn. Since there is no evidence that the mesencephalic sites that produce analgesia when stimulated electrically or by local injection of opiates project directly to spinal cord, it is likely that these mesencephalic regions exert their analgesic action indirectly on spinal cord through the nucleus raphe magnus. Consistent with this view is our preliminary finding that single neurons in NRM of the cat are strongly excited by intravenous morphine. This effect is reversed by naloxone, a specific opiate antagonist. It is possible that other pathways are involved in mediating the analgesia that is observed behaviorally. However, the reversal of morphine analgesia by NRM lesions (29) and the reversal of both morphine and stimulus-produced analgesia by lesions of the dorsolateral funiculus strongly implicate the NRM descending pathway. Furthermore, the reversal of both morphine and stimulus-produced analgesia by serotonin depletion (30–32) supports the hypothesis that the serotonin-containing nucleus raphe magnus neurons inhibit pain transmission.

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