Stimulation – Produced Antinociception from the Nucleus Tractus Solitarius : Involvement of the Periagueductal Gray Matter

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Electrical or chemical activation of the nucleus tractus solitarius (NTS) can produce antinociception in rats. This study investigated the functional involvement of midbrain structures, especially the periaqueductal gray matter (PAG), in antinociception from the NTS. The threshold for NTS stimulation – produced antinociception (SPA) was tested before and after the following lesions: 1) Knife sections at the rostral and/ or caudal limits of the midbrain; 2) Electrolytic lesions of the PAG; and 3) Spinal lesions. An increase in SPA threshold occured after sections caudal but not rostral to the PAG, after lesions in dorsal and lateral but not ventral PAG, and after complete spinal transection or selective bilateral section of the dorsolateral funiculus. It is suggested that antinociception triggered by activation of the NTS is mediated in part by NTS projections to the PAG.

INTRODUCTION

Activation of the nucleus tractus solitarius (NTS) by direct electrical stimulation or by glutamate microinjection can produce antinociception (Lewis et al., 1987; Morgan et al., 1989). Studies by Randich, Maixner and co – workers (see Randich & Maixner, 1984) suggest that inhibition of nociception mediated by the NTS is part of an integrated response to stress involving activation of vagal afferents to the

NTS. These authors have shown that stress – induced analgesia is attenuated by unilateral vagotomy (Maixner & Randich, 1984). Electrical stimulation of the vagus nerve produces antinociception , and this antinociception is reversibly blocked by lidocaine lesions of the NTS (Randich & Aicher, 1988). In addition, chemical or physiological activation of vagal afferents has been shown to inhibit nociceptive reflexes. Specifically, intravenous administration of D – ala^2 – methionine enkephalinamide (Randich & Maixner, 1984a), stimulation of cardiac receptors with veratrine (Randich et al., 1980), and volume expansion (Maixner & Randich, 1984a) all produce

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antinociception dependent on the integrity of vagal afferents. Moreover, nociceptive spinal neurons are inhibited by vagal stimulation (Ammons et al., 1983).

The efferent limb of this vagal - NTS antinociceptive system is relatively unexplored. A number of possible efferent pathways should be considered since NTS neurons make contact with many structures thought to be involved in the modulation of nociception. These structures include the periaqueductal gray matter (PAG) (Loewy & Burton, 1978; Bandler & Tork, 1987), the nucleus raphe magnus (NRM) and nucleus paragigantocellularis (Beitz, 1982), the nucleus reticularis gigantocellularis (Palkovitz & Zaborsky, 1977), the parabrachial nuclei (Leowry & Burton, 1978; Norgren, 1978), the locus coeruleus (Clavier, 1978; Savai et al., 1977), and the paraventricular nucleus (Ricardo & Koh, 1978; Swanson & Sawchenko, 1983). Direct projections to the spinal cord have also been shown (Loewy & Burton, 1978).

NTS connections with the PAG and NRM are particularly interesting since the involvement of these structures in antinociception is relatively well characterized (see Basbaum & Fields, 1984). Electrical and chemical activation of the PAG or NRM is known to inhibit nociception (Mayer et al., 1971; Zorman et al., 1982; Jensen & Yaksh, 1984). Recent evidence suggests that the NRM is part of an antinociceptive system involving the vagus nerve and NTS. Reversible inactivation of the NRM by microinjection of lidocaine attenuates antinociception produced by vagal stimulation (Randich & Aicher, 1988). The role of the NRM in antinociception from vagal stimulation appeares to be selective, however, in that inactivation of NRM does not disrupt antinociception produced by chemical activation of the vagus (Randich, Aimone, and Gebhart, 1987). The objective of the present study is to determine if the PAG, like the NRM, is involved in SPA from the NTS.

GENERAL MATERIALS AND METHODS

Male Sprague - Dawley rats (250 - 400 g) were anesthetized with pentobarbital (55mg/kg, i. p.) and mounted in a stereotaxic apparatus. The incisor bar was set 3.3mm below the interaural line to maintain the skull in a horizontal plane. The dorsal caudal medulla was exposed, and an electrode was lowered into the commissural region of the NTS at a 60 angle from the horizontal plane caudally.

Following surgery, nociceptive responsiveness was assessed using the tail - flick test (D'Amour & Smith, 1941) to noxious radiant heat. The tail - flick reflex is mediated at the spinal level and can be measured beginning approximately 40 min after an anesthetic dose of pentobarbital (Stein et al., 1987). Baseline nociceptive responsiveness was defined as the mean of three tail - flick latencies. Tail - flick trials were carried out until the mean was 4.5 second (s) or lower and no cases differed from the mean by more than 0.5s. Tail - flick inhibition was defined as a latency of 7s at which time the heat was automatically terminated to avoid tissue damage.

Following baseline testing, the NTS stimulation threshold for inhibition of the tail - flick reflex was determined. Electrical stimulation consisted of 50Hz monophasic pulses (0.4ms) which preceded by 20s and remained on during the tail - flick test. Current intensity was incrementally increased until the tail - flick reflex was inhibited, motor responses were elicited (e.g., movement of the jaw, tongue, whiskers), or brain stimulation current exceeded $500 \mu A$. All tests were separated by 1 min. Stimulation - produced antinociception of the tail - flick reflex (called stimulation - produced antinociception or SPA) was always followed by one or more tail - flick tests in the absence of brain stimulation until latency had returned to the baseline level. Determination of the threshold for SPA required that the tail - flick be in-

hibited twice at a given current intensity.

Once the threshold for SPA was determined, a CNS lesion was made. Following the lesion, tail‐flick latency and SPA threshold were determined again as above. Upon completion of testing, a lethal dose of pentobarbial (100 mg/kg, i.p.) was administered. The rat was then intracardially perfused with formalin and the brain revomed and sectioned for histological verification of electrode and lesion placements. Only rats with stimulation sites located within the NTS were included in the data analysis.

EXPERIMENT 1: CORONAL TRANSECTIONS

METHODS

Thirteen rats were prepared as above. Prior to placement of the NTS electrode, a hole was made 5 mm lateral to midline and either 5 mm rostral or 1 mm caudal to the interaural line. These holes allowed partial coronal sections of the brain to be made either rostral or caudal to the midbrain (Paxinos & Watson, 1982). A guide cannula was lowered through the hole into the brain. After establishing baseline tail‐flick latency and SPA threshold, a wire knife was passed through the guide cannula projecting 10 mm ventrolaterally. The entire assembly was then lowered and raised until the knife came in contact with the skull at the ventral and dorsal aspects of the brain, respectively. At least 10 min following transection, post‐lesion tail‐flick latency and SPA threshold were determined as before. In this experiment, NTS stimulation consisted of anodal pulses delivered through a monopolar electrode. Maximum current intensity for NTS stimulation never exceeded 500 μA.

RESULTS

Analysis of variance revealed a significant effect of brain lesions on SPA threshold ($F = 9.03$, $p <$

.01). Rats receiving lesions caudal to the midbrain showed a significant increase in SPA threshold (M$\pm$SEM; 350$\pm$39.1 μA) compared to pre‐lesion threshold (M$\pm$SEM; 159.4$\pm$16.7 μA) and compared to the SPA threshold (M$\pm$SEM; 193.8$\pm$32.2 μA) of rats receiving lesions rostral to the midbrain (Tukey, $p <$.01; Figure 1). The post‐lesion SPA thresholds for rats receiving rostral lesions (M$\pm$SEM; 193.8$\pm$32.2 μA) did not differ significantly from the pre‐lesion values (M$\pm$SEM; 137.5$\pm$11.3 μA). No difference in SPA threshold for the two groups was evident prior to lesions.

SPA thresholds were determined in 3 rats following successive rostral ans caudal lesions. Although little or no change in threshold occurred following the initial rostral lesion, caudal transection produced an increase in threshold of at least 100% in all 3 rats.
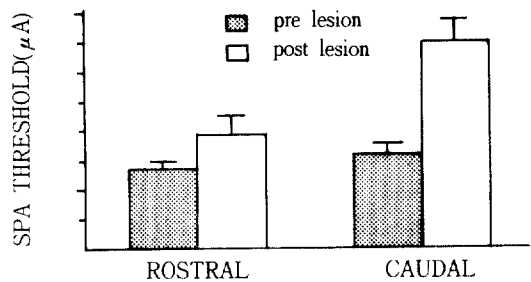

Figure 1: **Mean SPA threshold before and after transection of the brain rostral and caudal to the PAG.**

EXPERIMENT 2: PAG LESIONS

METHODS

Thirty‐six rats were prepared as above except that a monopolar electrode was lowered into the PAG immediately prior to placement of a bipolar stimulating electrode in the NTS. Electrolytic lesions of the PAG were produced by passing 1‐
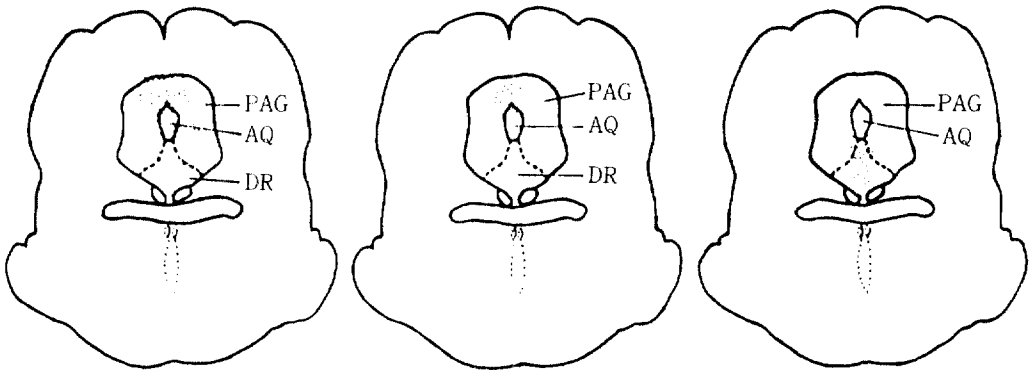
Figure 2: Representative large, dorsal, and ventral electrolytic lesions of the PAG. All sections are 1.2mm from lambda (Paxinos & Watson, 1982).

2mA direct anodal current through the monopolar electrode for 10 - 15s. The circuit was completed by clipping the cathode to the rat's ear. Three different lesion groups were defined based on histological verification of the lesion: 1) A ventral lesion group (N = 12) received lesions in and around the dorsal raphe nucleus of the ventral medial PAG; 2) A dorsal lesion group (N = 15) had tissue damage restricted to dorsal and lateral aspects of the PAG; and 3) A group of rats (N = 7) with tissue damage encompassing both dorsal and ventral parts of the PAG. Representative lesions for these three groups are presented in Figure 2. Threshols for SPA from the NTS were determined before and after the lesion and never exceeded $200 \mu A$.

RESULTS

ANOVA revealed a significant effect of PAG lesion on SPA threshold ($F = 30.4$, $p < .01$). Post hoc analysis showed significant increses in SPA threshold following lesions in the dorsal and dorsal/ventral PAG lesion groups, but not in the ventral PAG lesion group, compared to pre - lesion thresholds for each group (Tukey, $p < .01$; Figure 3). Although no group differences in threshold were evident prior to lesions, rats receiving dorsal lesions

had SPA thresholds that were significantly greater than the thresholds for rats receiving ventral (Tukey, $p < .01$). Rats receiving considerably larger combined dorsal/ventral lesion had significantly greater SPA thresholds than rats with either specific dorsal or ventral lesions (Tukey, $p < .05$).
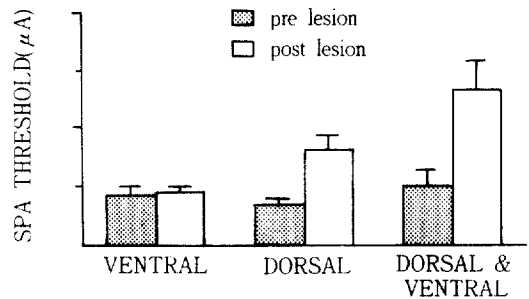

Figure 3: Mean SPA threshold before and after electrolytic lesions of ventral, dorsal, and both regions of the PAG.

EXPERIMENT 3: SPINAL LESIONS

Brainstem nuclei known to be involved in antinociception inhibit nociceptive reflexes at the spinal level (Basbaum & Fields, 1984). The dorsolateral

funiculus (DLF) appears to be a critical spinal pathway in desending modulation since specific DLF lesions attenuate antinociception from electrical or chemical stimulation of the PAG (Basbaum et al., 1977; Murfin et al., 1976). In the present study, complete spinal transection and selective DLF lesions were made to determine if similar descending systems modulate nocicepetion from the NTS.

METHODS

Six rats were anesthetized and prepared as above except that a laminectomy was performed at the T 7 - 8 level prior to exposure of the dorsal caudal medulla. A bipolar electrode was then positioned in the NTS. Baseline pain sensitivity and the threshold for SPA were determined as described in the General Method. The spinal cord was then transected at the T 7 level. Upon recovery of the tail‐flick (approximately 60min later), the rat was re‐tested as above begining with the SPA threshold intensity previously determined. NTS stimulation was then increased progressively until tail‐flick inhibition occurred, current intensities reached $300\,\mu A$, or motor effects (e. g., whisker movements) were elicited.

The effect of specific DLF lesions was tested in an additional 16 rats. Rats were anesthetized and prepared as above except that the first two cervical vertebrae were removed prior to implantation of a bipolar electrode in the NTS. Tail‐flick latencies and thresholds for SPA were determined before and after partial sections of the lateral spinal cord at the C2 level as described above.

RESULTS

Complete spinal transection resulted in a significant reduction in baseline tail‐flick latency (($M\pm$ SEM before and after transection $= 4.0\pm0.2$ and 3.2 ± 0.2s; paired $t = 4.50$, $p < .01$). The mean$\pm$SEM threshold for SPA prior to transection was 55 ± 8.9

μA. Spinal transection completely abolished SPA in all 6 rats (i. e., current intensities reached $300\,\mu A$ or produced motor effects with no change in tail‐flick latency).

Rats receiving lesions in the lateral spinal cord were placed in one of two groups based on the extent of bilateral damage to the DLF. Substantial bilateral lesions of the DLF were histologically verified for 10 of the 16 rats. These rats had a significantly lager percent increase in SPA threshold following the lesion than rats ($N = 6$) with only partial lesions of the lateral spinal cord ($M\pm$SEM ; $137\pm39\%$ and $11\pm12\%$; $t = 2.4$, $p < .05$).

DISCUSSION

These data demonstrate that the antinociceptive effect of NTS stimulation is dependent, at least in part, upon the midbrain. Transection caudal to the midbrain causes large increases in SPA threshold from the NTS, whereas transection rostral to the midbrain is much less disruptive. The dorsal and lateral PAG appear to be critical midbrain regions in NTS antinociception since selective lesions of these areas attenuate NTS SPA. The PAG and NTS appear to share a common spinal pathway in inhibiting the tail‐flick reflex; complete spinal transection abolishes NTS SPA, and selective lesions of the dorsolateral funiculus more than double the current necessary to inhibit the tail‐flick reflex.

Antinociception has been produced by actvation of neural structures located at all levels of the brain. These structures include the frontal cortex (Hardy, 1985), lateral hypothalamus (Carr & Uysal, 1985), and habenula (Cohen & Melzack, 1985), in addition to various brainstem nuclei (Basbaum & Fields, 1984). Although forebrain control of brainstem nuclei involved in the modulation of nociception is surely present, it does not appear to be critical for the NTS antinociceptive system to be activated. Antinociception produced by activation of the NTS is unaffected

in rats with partial transection of the brain immediately rostral to the midbrain. In contrast, partial transection caudal to the midbrain attenuates the antinociceptive effect of NTS stimulation. This region of the midbrain, thus, appears be functionally involved in NTS SPA.

A number of midbrain structures have been shown to support SPA. These include the PAG (Mayer et al., 1971), parabrachial nuclei, and ventral tegmental area (Sarkis et al., 1984). The PAG is of particular interest since it receives NTS efferents (Bandler & Tork, 1987) and has been studied extensively with regard to antinociception. In aggreement with Bandler & Tork (1987) work in the cat showing dorsolateral termination of NTS efferents to the PAG, it was found that dorsal and lateral lesions of the PAG attenuate NTS SPA. Lesions of the ventral PAG, specifically in and around the dorsal raphe nucleus, had no effect on antinociception from the NTS. This finding is somewhat surprising given that the ventral PAG has been characterized as supporting "pure analgesia" (Fardin et al., 1984b), whereas the antinociception from activation of the dorsal and lateral PAG has been suggested to be secondary to aversive reactions (Fardin et al., 1984a). Although dorsal and lateral PAG stimulation is known to be aversive, antinociception can be produced from this region in the absence of aversive reactions (see Morgan, 1989). Moreover, NTS SPA, which depends at least in part on the dorsal and lateral PAG, can be measured in awake rats in the absence of aversive or motor responses (Sohn et al., 1988).

The PAG is not the only structure mediating the antinociceptive effect of NTS stimulation. SPA from the NTS can be produced, althought at significantly higher intensities, even following large lesions of the PAG. Moreover, Randich and Aicher (1988) found that reversible lidocaine lesions of the nucleus raphe magnus – medullary reticular formation or lateral reticular nucleus attenuate analgesia from stimulation

of the vagus nerve. It thus appears that the PAG, ventral medial medulla, and lateral reticular nucleus are all called into play by activity in vagal afferents relaying in the NTS. In addition, the NTS has efferent projections to other brainstem areas implicated in antinociception. These areas include the nucleus paragigantocellularis (Beitz, 1982), the nucleus reticularis gigantocellularis (Palkovitz & Zaborsky, 1977), the parabrachial nucleus (Leowry & Burton, 1978; Norgren, 1978), the locus coeruleus (Clavier, 1978; Sabai et al., 1977), and the paraventricular nucleus (Ricardo & Koh, 1978; Swanson & Sawchenko, 1983).

Vagal afferents to the NTS appear to form the afferent component of a feedback system modulating nociception. This feedback system reports alterations within the viscera to the NTS where pain inhibitory systems can be activated through NTS efferents (see Randich & Aicher, 1988). Specifically, NTS efferents to the dorsal and lateral PAG appear to function in this capacity. The nucleus raphe magnus and lateral reticular nucleus also seem to be involved since antinociception from vagal stimulation, which seems to relay in the NTS (Randich & Aicher, 1988), is attenuated by lidocaine lesions of these regions. The descending pathway for this system appears to lie principally within the DLF, as shown in this study and by numerous others investgating antinociceptive properties of the PAG and NRM (see Basbaum & Fields , 1984).

These findings are another step toward delineating antinociceptive pathways within the nervous system. Although many brain regions appear to be involved in nociception modulation, the functional relationalship of these regions is relatively unknown. Moreover, this research and that of Randich and co - workers, provide insight into the natural activation of this antinociceptive system.

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