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Effects of Raphe Magnus and Raphe Pallidus Lesions on Morphine-Induced Analgesia and Spinal Cord Monoamines

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PROUDFIT, H. K. *Effects of raphe magnus and raphe pallidus lesions on morphine-induced analgesia and spinal cord monoamines.* PHARMAC. BIOCHEM. BEHAV. 13(5) 705–714, 1980.—These studies examined the role of bulbospinal serotonin-containing neurons found in the nucleus raphe magnus and nucleus raphe pallidus in the mediation of morphine-induced antinociception. Lesions were made using both electrolytic coagulation and the axon-sparing technique of monosodium-L-glutamate injection to ascertain whether the effects following lesions in the area of the medullary raphe nuclei are due to destruction of neuronal perikarya or fibers passing near these nuclei. These studies revealed that lesions of both the raphe magnus and raphe pallidus resulted in decreased nociceptive thresholds and attenuation of morphine-induced analgesia. Such effects were observed regardless of the lesioning method used, which suggests that destruction of neurons in these nuclei was responsible for lesion-induced effects. In addition, lesion-induced changes in spinal cord serotonin content and morphine analgesia were significantly correlated which lends support to the conclusion that the bulbospinal serotonin systems are necessary for the mediation of morphine effects. Furthermore, no correlation was observed between changes in spinal cord norepinephrine content and morphine analgesia. This observation suggests that lesion-induced damage to bulbospinal noradrenergic fibers which pass near the midline does not contribute to the attenuation of morphine analgesia resulting from raphe lesions.

Analgesia Raphe nuclei Serotonin Norepinephrine Glutamic acid Morphine

THE mesencephalic periaqueductal gray region (PAG) has been shown to be a major site necessary for the induction of antinociception by opiate-like substances. Both electrical stimulation [33, 38, 39] and microinjection of opiates [28, 71, 73] in the PAG induce analgesia. These effects appear to be mediated by a spinal action since both electrical stimulation [25, 34, 43] and local injection of opiates into the PAG [9] block the activity of spinal cord lamina V cells evoked by noxious peripheral stimulation. However, the PAG lacks significant connections with the spinal cord [5, 29, 30], but does project to the nucleus raphe magnus (RM) of the caudal brain stem [24]. Several lines of evidence suggest that the RM is an important component in the neuronal circuitry mediating opiate-induced analgesia. For example, the projection from PAG to RM has been demonstrated to have functional significance since electrical stimulation of the PAG [35] or the local application of morphine to this area [7] result in the activation of a certain population of RM neurons. It should be noted, however, that the effects of morphine were not particularly potent [7]. The systemic administration of morphine also produces an increase in the firing frequency of raphe magnus neurons [2, 20, 42]. Thus, morphine may act by increasing the firing rate of PAG neurons which in turn activate RM neurons. This suggestion is supported by reports demonstrating that electrical stimulation of the RM is capable of inducing potent analgesia [44, 45, 52]. Furthermore, electrical stimulation of the RM produces a powerful inhibition of spinal cord dorsal horn neurons activated by noxious cutaneous stimulation [3, 22, 23, 26] and identified spinothalamic tract neurons [6, 40, 68]. These data lead to the suggestion that RM stimulation induces analgesia by inhibiting neuronal circuits in the spinal cord activated by noxious peripheral stimulation.

Analgesia has also been reported following the microinjection of morphine in the RM [31, 32, 49], although others have not observed such actions [1, 60, 61]. These conflicting results may be due to differences in analgesiometric testing procedures and the doses used. Thus, in addition to being an important relay in the pathway from the PAG to the spinal cord, the RM may also be a site of action for the induction of analgesia by opiates.

The RM includes some serotonin-containing (5-HT) perikarya which project to the spinal cord dorsal horn [12,18]. It has been suggested that the antinociceptive effects of RM activation are due to the release of 5-HT from these bulbospinal projections (see Messing and Lytle [41] for review). One line of evidence which supports this proposal is the demonstration that lesions in the raphe area attenuate opiate-induced analgesia. However, studies relating the effects of RM lesions to opiate-induced analgesia have not included determinations of spinal cord 5-HT following such lesions [13, 52, 70]. Thus, the role of 5-HT in the reported lesion effects is not clear. The major objective of the present study was thus to examine the correlation between raphe

magnus lesion-induced alterations in spinal cord 5-HT and alterations in opiate-induced analgesia. Consideration was also given to the specificity of raphe magnus lesions in producing changes in the analgesic actions of morphine. More specifically, lesions were made using both electrolytic coagulation and the axon-sparing lesioning technique of glutamic acid injection [46, 58, 64] in an attempt to ascertain whether the effects following lesions in the area of the RM are due to destruction of cell perikarya or fibers passing near the RM. In addition, lesions of both types were also made in the nucleus raphe pallidus. This additional site was studied since it too is a 5-HT-containing nucleus with spinal projections similar to those of the RM [10, 17, 67].

METHOD

Lesioning Procedures

Female Sprague-Dawley derived rats weighing between 250 and 280 g were pretreated with atropine sulfate (1 mg/kg) to prevent bronchial secretions and anesthetized with ether. Following loss of the righting reflex the animals were placed in a stereotaxic frame and lesions were made in either the nucleus raphe magnus or the nucleus raphe pallidus. The stereotaxic coordinates for these sites are as follows: Raphe magnus: 3.0 mm posterior to the interaural line; 1.0 mm below the interaural line; 0.00 mm lateral to the midline. Raphe pallidus: 4.0 mm posterior to the interaural line; 1.0 mm below the interaural line; 0.00 mm lateral to the midline. The incisor bar was placed 2.5 mm below the interaural line. Electrolytic lesions were made in these nuclei by passing anodal D.C. current between the uninsulated tip of a 25 ga wire and a copper rectal indifferent electrode. Electrical current was generated using a Grass S4 stimulator and constant current unit (CCU-1). Lesions approximately 2 mm in diameter were made by passing 2 mA for 10 sec while lesions of 0.5 mm in diameter resulted from current of 1 mA for 5 sec. Other animals received lesions made by microinjection of monosodium-L-glutamic acid as described by Simson and coworkers [58]. Glutamate injections were made in ether-anesthetized animals using a stereotaxically-placed injection tube (28 ga) connected to a gear-driven 10 μl syringe by a 20 cm length of polyethylene tubing (PE 20). Each site was injected with 5 μl of 1% glutamate infused at a rate of 0.5 μl/min. The flow of solution was verified by monitoring the movement of a bubble in the polyethylene tubing. The cannula was left in place for 5 minutes following the injection to prevent the escape of material up the cannula track. All lesioned animals were allowed two weeks for recovery from surgery before their response to morphine was tested. Control rats were the same age and sex as the lesioned rats. During the entire course of the experiment all animals were housed in groups of 5 and allowed free access to food and water.

Analgesiometric Testing

Antinociception induced by morphine in lesioned and non-lesioned control animals was assessed using the tail flick assay [19]. The tail flick response was elicited by the application of a focused beam of high intensity light on various parts of the rat's tail which had been previously blackened to allow uniform absorption of heat. The time interval between the onset of the light stimulus and the tail flick response was measured electronically and terminated at 14 sec in the absence of a response. The tail flick latency (TFL) was defined as the average of three determinations taken in immediate succession. Animals were tested prior to lesioning and both preceding and following each dose of morphine which was given two, three, and four weeks following surgery.

Testing Schedule

These studies consisted of one main experiment (1) plus two other experiments (2 and 3) which were designed to provide additional data points which were missing in the main experiment due to misplaced lesions and deaths. These experiments were conducted as follows:

Experiment 1. Ninety rats were divided into four experimental groups (20 rats per group) and a control group (10 rats), and pre-lesion tail flick latencies determined. Lesions were placed in the raphe magnus and raphe pallidus using either electrolytic coagulation or glutamic acid injections. Ten of the animals in each of the two electrolytic lesion groups received small lesions and 10 received large lesions. Fourteen days after surgery tail flick latencies were again determined and morphine was given immediately thereafter. Tail flick latencies were determined 30 min following morphine injection. Morphine sulfate was dissolved in 0.9% saline and administered subcutaneously to non-lesioned control animals in doses of 1.0, 2.5, and 5.0 mg/kg and to lesioned animals in doses of 5.0, 7.5, and 10.0 mg/kg in a constant volume of 0.1 ml/100 g body weight. The doses were given in ascending order beginning with the lowest dose at 14 days following surgery. Subsequent doses were given at seven day intervals.

Experiment 2. This experiment was designed to provide more data for the relationship between destruction of the raphe magnus and the capacity of morphine (5 mg/kg, SC) to induce analgesia. For this experiment, 20 rats were lesioned using electrolytic coagulation of the raphe magnus. Fourteen days after surgery tail flick latencies were obtained, morphine (5 mg/kg) was given, and tail flick latencies measured 30 min later.

Experiment 3. This experiment provided data for sham-lesioned controls and for additional animals with either glutamic acid or electrolytic lesions in the raphe pallidus to test lesion-induced effects on tail flick latency. Twenty-five rats were tested using the tail flick test and then five of these were lesioned with glutamic acid and 10 with electrolytic coagulation. The surgical procedures for the sham-lesioned animals were identical to those used with the lesioned animals except no current was passed. Fourteen days after surgery tail flick latencies were again determined and the animals were killed and the lesion placements verified histologically.

Histological Procedures

Following completion of each experiment the animals were killed by decapitation and each brain removed and fixed in a solution of 10% Formalin and 30% sucrose in 0.9% saline. The spinal cord from each animal was rapidly removed and processed for the determination of monoamine levels. The brains were cut in the coronal plane and 20 μ sections were taken at 100 μ intervals and stained with cresyl violet. Drawings were made from the projected image for each section in which lesion damage appeared. These sections were compared with an atlas constructed from normal brain sections taken at 100 μ intervals. Neuronal structures

in these sections were identified using descriptions of the caudal brain stem provided by Valverde [62,63], Palkovits and Jacobowitz [47], Dahlstrom and Fuxe [17,18], and Pellegrino and Cushman [48]. Estimates of the extent to which various structures sustained damage were made by measuring the area of destruction by planimetry and expressing this value as a percent of the total area of the structure in each section. The degree of destruction was estimated for each section in which damage appeared and the average of the percent destruction in all sections was used as an index of the total damage in each brain.

Monoamine Assay Procedures

Monoamine concentrations in the spinal cord caudal to and including the lumbar enlargement were determined at the conclusion of the experiment. Ten rats were randomly selected from each of the four experimental groups; five rats from each group of ten were used for 5-HT and five for norepinephrine (NE) assays. NE was determined spectrofluorometrically using a modification of the methods described by Welch and Welch [66] for NE extraction and the fluorophore development was done according to Chang [14]. Serotonin determinations were made using the method of Welch and Welch [66] for extraction of 5-HT from the spinal cords and the method of Maickel and Miller [36] for the fluorophore development using o-phthalaldehyde. Monoamine concentrations in spinal cords taken from the 10 unoperated control animals plus 14 naive rats were determined simultaneously with those of lesioned animals. Serotonin (12 samples) and norepinephrine (12 samples) content in 24 control animals was 774.2 ± 77.1 ng/g and 300.7 ± 19.4 ng/g, respectively.

Statistical Procedures

Prior to statistical analysis all tail flick latency (TFL) measurements were converted to a ratio of the increase in TFL after morphine to the maximum possible increase. Such a ratio eliminates the variability due to differences in control (Pre-drug) TFLs and allows more valid comparisons among animals. This ratio, called the Analgesia Index (AI), was calculated as follows:

$$AI = \frac{\text{TFL after morphine} - \text{control TFL}}{\text{Cut-off time (14 sec)} - \text{control TFL}}$$

An AI of 1.00 indicates maximum antinociception, 0.00 no effect, and negative values indicate hyperalgesia.

Alterations in pain sensitivity and dose-related effects of morphine following lesions were assessed using a one-way analysis of variance and comparisons of the various group means with the control group mean was done using the Newman-Keuls test for multiple comparisons [69]. Correlations between alterations in morphine-induced analgesia and spinal cord monoamine levels and between morphine-induced analgesia and the percent destruction of the raphe nuclei were determined using the Spearman Rank Correlation method [56].

RESULTS

Lesion Sites

Lesions made in the nucleus raphe magnus and nucleus raphe pallidus using both electrolytic coagulation and the local injection of monosodium-L-glutamic acid are illustrated in Fig. 1. Electrolytic lesions of two different sizes were made to assess the effect of large and small lesions on the capacity of morphine to induce analgesia. Lesions produced by passing currents of 1 mA for five seconds or 2 mA for 10 sec were approximately 0.5 mm and 2.0 mm in diameter, respectively. The small lesions resulted in approximately 30% destruction of the raphe with very slight damage to lateral structures while the large lesions destroyed the entire raphe area including a significant area lateral to the midline. With large raphe magnus lesions, the medial third of the nucleus gigantocellularis was damaged while large raphe pallidus lesions included much of the nucleus reticularis paramedianus.

By contrast, lesions resulting from local injections of glutamate were much smaller and in most cases did not extend into the medial reticular formation to any significant degree. These lesions appeared as cylindrical or teardrop-shaped regions of intense gliosis with a complete lack of neuronal perikarya. The size of the lesion at the injection site was usually between 0.5 and 0.7 mm in diameter and one to two mm long. The elongated shape of the gliosis apparently resulted from the flow of the glutamate solution up the channel made by the injection cannula. Blood vessels in the vicinity of the lesion appeared normal and in some cases were seen to course through the middle of the lesion. Cavitation of brain tissue due to the volume of solution injected was never seen; the only evidence of destruction produced by physical forces was the track produced by the insertion of the injection cannula.

A given animal was included in one of the four lesion groups in Experiment 1 if 20% or more of the target nucleus had been destroyed. Thus, of the 65 rats surviving surgery, 14 had electrolytic and 11 had glutamate lesions of the raphe magnus while 5 had electrolytic and 8 had glutamate lesions of the raphe pallidus. The remaining 27 rats had lesions which included less than 20% of either the raphe magnus (6 electrolytic and 5 glutamate) or the raphe pallidus (7 electrolytic and 4 glutamate), and five rats had lesions which included parts of the n. reticularis paramedianus (3 electrolytic and 2 glutamate). In Experiment 2, 15/20 rats survived the lesioning procedure and 9 of these sustained damage greater than or equal to 20% of the raphe magnus. In Experiment 3, 6/10 electrolytically-lesioned and 2/5 glutamate-lesioned rats sustained damage to the raphe pallidus equal to or greater than 20%.

Behavioral Effects of Raphe Lesions

Most animals receiving small raphe lesions did not exhibit any behavioral alterations or motor deficits. However, some of the animals with large lesions were somewhat ataxic, but none showed impairment of the tail flick response. Since none of the animals receiving raphe lesions was judged to be severely impaired, either behaviorally or motorically, all of the lesioned animals were used in these studies.

Effect of Lesions on Nociceptive Threshold

The capacity of lesioned animals to respond to noxious thermal stimulation was assessed by comparing the tail flick latency determined before surgery with that two weeks following surgery. Table 1 shows pre- and post-lesion TFLs for unoperated control and sham-lesioned animals, as well as animals receiving raphe magnus and raphe pallidus lesions made using either electrolytic coagulation or the injection of

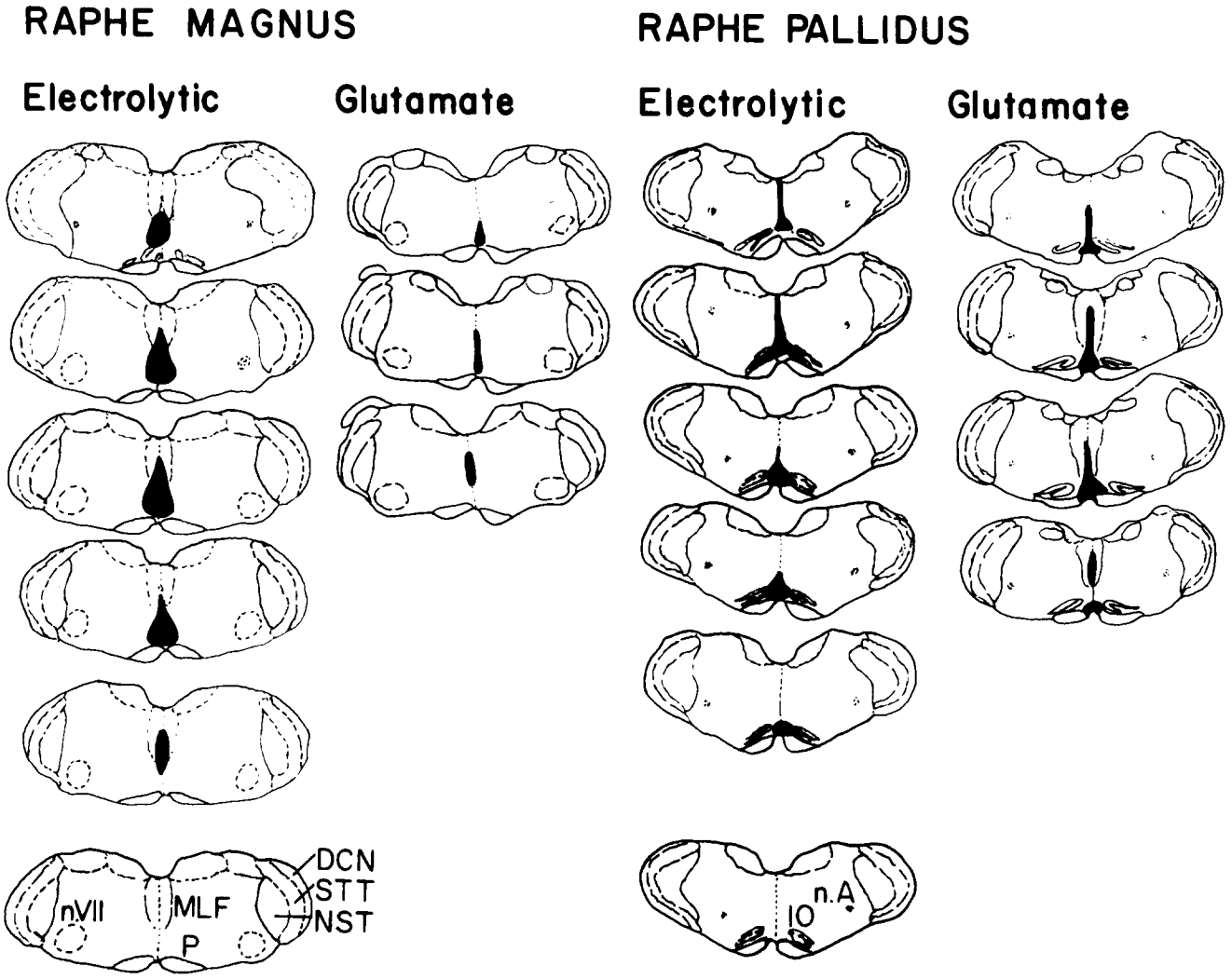

FIG. 1. Representative lesion sites that were effective in reducing the antinociception induced by morphine. Lesions on the left were produced by electrolytic coagulation or the microinjection of monosodium-L-glutamic acid in the raphe magnus, while those on the right were made in the raphe pallidus. The coronal sections shown are reproductions of drawings made from projected brain sections. The interval between sections is 100 micra. Abbreviations: DCN, dorsal cochlear nucleus; IO, inferior olive; MLF, medial longitudinal fasciculus; nA, nucleus ambiguus; nVII, nucleus of the facial nerve; NST, nucleus of the spinal trigeminal tract; P, pyramid; STT, spinal tract of the trigeminal nerve.

glutamate. The mean pre- and post-lesion tail flick latencies for the 10 unoperated control animals were not significantly different ($p>0.05$, t test) from those for the 10 sham-lesioned controls. These two control groups were pooled and the mean tail flick latencies are shown in Table 1. The data illustrated in Table 1 were derived primarily from animals in Experiment 1 which exhibited lesions equal to or greater than 20% of the target nucleus. Also included in Table 1 are data from the six rats with electrolytic lesions and the two with glutamate lesions of the raphe pallidus from Experiment 3 which exhibited destruction equal to or greater than 20%. Table 1 also shows the analgesia indices (AI) calculated by dividing the difference between pre- and post-lesion TFLs by the maximum possible TFL (14 sec—pre-lesion TFL). Comparisons were made between each lesion-group mean AI and the control mean AI using the Newman-Keuls test for multiple comparisons. The mean square error term in the Newman-Keuls analysis was derived from a one-way analysis of variance which indicated a statistically significant effect ($p>0.01$) of lesions on tail flick latency. Individual comparisons indicate that all four experimental group means were statistically different ($p<0.01$) from the mean AI for the control group. Furthermore, the positive AI value for the control group indicates a slight decrease in sensitivity to noxious stimulation, while the negative values for the four lesion groups indicate increased sensitivity resulted from lesions of the raphe magnus and raphe pallidus.

Morphine Dose-Response Relationship

The capacity of various doses of morphine to prolong the tail flick latency in 10 unoperated control and 38 raphe-lesioned animals is illustrated in Figs. 2 and 3. The data shown in these figures were obtained from the 38 rats used in Experiment 1 which had electrolytic lesions including 20% or more of the raphe magnus (14 rats) and raphe pallidus (5 rats)

TABLE 1

EFFECT OF RAPHE LESIONS ON TAIL FLICK LATENCY (TFL)

Lesion group	Pre-lesion* TFL (sec)	Post-lesion* TFL (sec)	Difference† (sec)	Analgesia index‡	n§
Non-lesioned and sham-lesioned controls	3.1 ± 0.3	4.9 ± 0.4	1.9 ± 0.3	0.18 ± 0.04	20
Electrolytic					
RM	3.2 ± 0.2	2.0 ± 0.2	−1.2 ± 0.2	−0.12 ± 0.02¶	14
RP	3.1 ± 0.2	2.3 ± 0.2	−0.8 ± 0.2	−0.08 ± 0.02¶	11
Glutamate					
RM	3.9 ± 0.3	2.5 ± 0.2	−1.5 ± 0.4	−0.16 ± 0.04¶	11
RP	2.9 ± 0.5	1.8 ± 0.1	−1.1 ± 0.5	−0.12 ± 0.06¶	10

*Mean TFL ± SEM.

†Difference between pre- and post-lesion tail flick latency (TFL) for each animal averaged over the entire group.

‡Analgesia indices represent the average for each group of animals.

§Number of animals in each group.

¶Indicates statistically significant difference ($p<0.01$) between group mean and control mean assessed using Newman-Keuls test for multiple comparisons.

Abbreviations: RM, raphe magnus; RP, raphe pallidus.

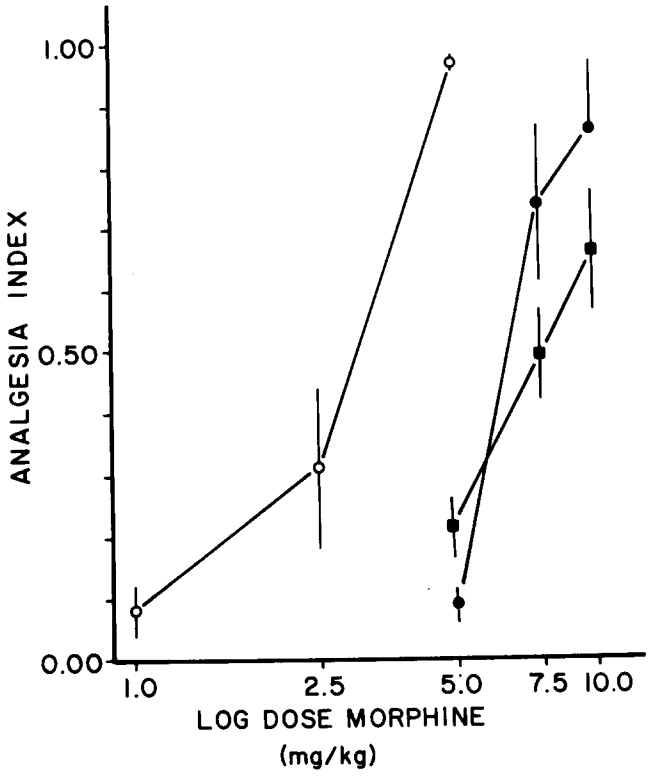

FIG. 2. Effect of electrolytic- and glutamate-induced lesions in the raphe magnus on the dose-response relationship for morphine. Open circles: unoperated control group; filled circles: electrolytic lesions; filled squares: glutamic acid lesions. The analgesia index is plotted on the ordinate and the log dose is plotted on the abscissa. Each point represents the mean analgesia index ± SEM.

or glutamate-induced lesions including 20% or more of the raphe magnus (11 rats) and raphe pallidus (8 rats). In Fig. 2 the dose-response relationship for morphine given to unoperated control animals is compared with that for morphine given to animals with raphe magnus lesions produced either electrolytically or by the injection of glutamate. The marked shift to the right in the dose-response curves for morphine given to lesioned animals indicates that the lesions attenuated the antinociceptive actions of morphine. Mean analgesia index values for 5.0 mg/kg of morphine given to unoperated control animals were compared with those for the same dose of morphine given to lesioned animals. These comparisons, made using the Newman-Keuls test, revealed that raphe magnus lesions resulted in a statistically significant ($p<0.01$) attenuation of morphine-induced antinociception. Figure 3 illustrates similar comparisons for raphe pallidus lesions. These data indicate that raphe pallidus lesions also significantly ($p<0.01$) reduced the capacity of morphine to prolong the tail flick latency.

Effect of Lesion Size on Morphine-Induced Antinociception

Figure 4 illustrates the relationship between the capacity of morphine (5 mg/kg) to induce antinociception and the amount of destruction produced by electrolytic and glutamate lesions of the raphe magnus and raphe pallidus. The data illustrated in Fig. 4 were derived from the 60 surviving raphe-lesioned rats from Experiment 1 and the 15 rats which survived in Experiment 2. The animals in Experiment 3 were not included since they did not receive morphine. These data were analyzed using the Spearman Rank Correlation test which revealed a statistically significant correlation ($p<0.01$) between the amount of damage to the raphe nuclei and the degree to which morphine-induced antinociception was attenuated. Such a correlation was found when lesions of both the raphe magnus and raphe pallidus were produced by either the electrolytic method or the injection of glutamate. Furthermore, it is apparent from Fig. 4 that lesions of either

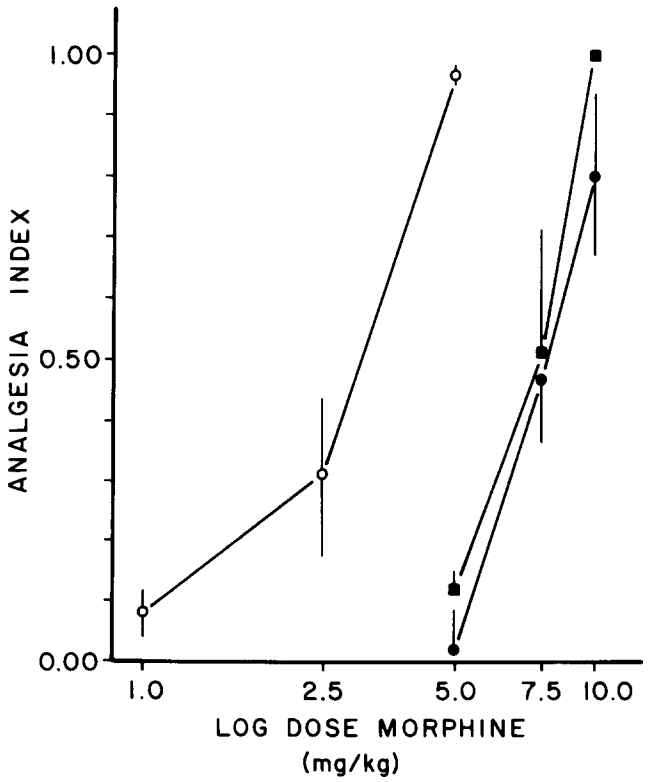

FIG. 3. Effect of electrolytic and glutamate-induced lesions in the raphe pallidus on the dose-response relationship for morphine. The symbols used are the same as those in Fig. 2.

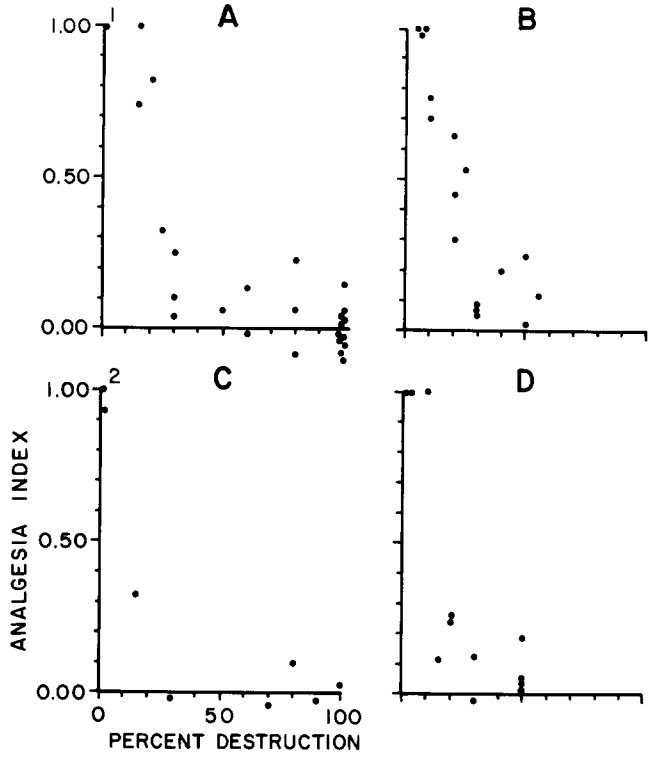

FIG. 4. Correlation of lesion size and degree of analgesia following morphine sulfate (5 mg/kg, SC). The ordinate represents the analgesia index and percent destruction of raphe nuclei is plotted on the abscissa. (A) Electrolytic lesions in the raphe magnus (n=35); (B) Glutamic acid lesions in the raphe magnus (n=16); (C) Electrolytic lesions in the raphe pallidus (n=12), (D) Glutamic acid lesions in the raphe pallidus (n=12). Spearman Rank Correlation Coefficients for these data were =0.91 ($p<0.01$), −0.90 ($p<0.01$), −0.69 ($p<0.01$), and −0.77 ($p<0.01$), respectively. The dots labelled 1 and 2 represent 10 and 5 rats, respectively.

type in either raphe nucleus produce a nearly complete blockade of 5 mg/kg of morphine if the lesion destroyed at least 30% of these nuclei.

Effect of Lesions on Spinal Cord Monoamine Content

Additional evidence for the role of 5-HT in the mediation of raphe lesion effects was obtained by examining the relationship between lesion-induced changes in spinal cord 5-HT levels and the capacity of morphine (5 mg/kg) to increase tail flick response latencies. Sixty-five rats survived the lesioning procedures in Experiment 1 and 40 of these were randomly selected for serotonin and norepinephrine assays. Three of the 20 samples used for the serotonin assay and 4 of the 20 samples used for the norepinephrine assay were lost during the assay procedures. Figure 5 shows that lesions of both the raphe magnus and raphe pallidus resulted in changes in spinal cord 5-HT levels. In addition, there is an inverse relationship between percent reduction of spinal cord 5-HT content and the capacity of morphine to induce analgesia. That is, lesions that produce small decreases in spinal cord 5-HT attenuate morphine effects slightly while larger decreases result in greater attenuation. Analysis of these data using Spearman's Rank Correlation method revealed a statistically significant inverse correlation ($r_s = -0.61$; $p<0.01$) between lesion-induced changes in spinal cord 5-HT and the corresponding analgesia index.

Norepinephrine (NE) terminals and fibers have been demonstrated near the raphe nuclei using fluorescence histochemical visualization [15, 18, 59]. Since bulbospinal NE systems have been implicated in the mediation of morphine analgesia [55], it is possible that destruction of those NE fibers which pass near the raphe nuclei may be responsible for some or all of the effects observed following raphe lesions. Such a possibility was examined by correlating lesion-induced changes in spinal cord NE with alterations in the capacity of morphine to induce analgesia. Most of the lesions were placed in the caudal raphe nuclei (magnus and pallidus), but some were found to have been inadvertently placed in the nucleus reticularis paramedianus, approximately 1 mm lateral to the raphe pallidus. These lesions all produced alterations in both spinal cord NE content and morphine-induced analgesia. If NE fibers passing near the midline raphe nuclei are involved in the mediation of the antinociception following morphine administration, there should be an inverse correlation between these two dependent variables. However, no correlation could be demonstrated (Fig. 6) between spinal cord NE content and morphine analgesia ($r_s = 0.05$; $p>0.05$).

DISCUSSION

Lesions of the nucleus raphe magnus have been previously reported to enhance responsiveness to noxious stimu-

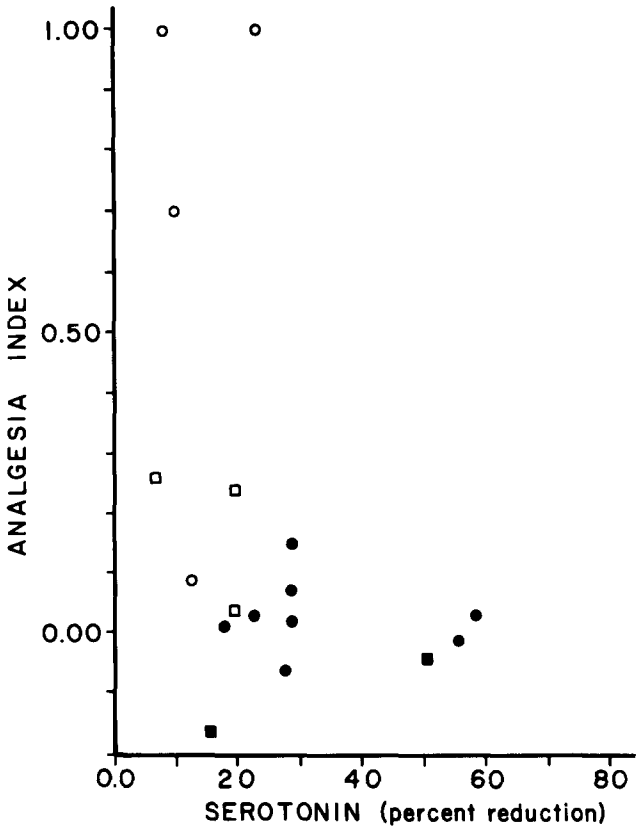

FIG. 5. Effect of raphe lesion-induced changes in spinal cords levels of serotonin on the capacity of morphine (5 mg/kg) to induce analgesia. Ordinate: analgesia index. Abscissa: percent reduction of serotonin in the caudal half of the spinal cord. Spearman Rank Correlation Coefficient=−0.60 ($p<0.01$). ●, electrolytic lesions of the raphe magnus (n=8); ○, glutamate lesions of the raphe magnus (n=4); ■, electrolytic lesions of the raphe pallidus (n=2); □, glutamate lesions of the raphe pallidus (n=3).

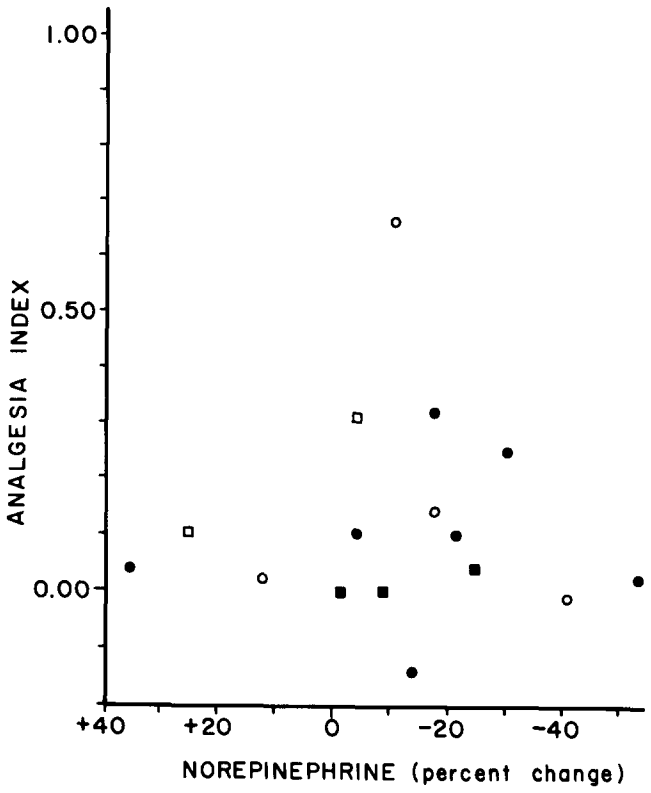

FIG. 6. Effect of midline and paramidline lesions of the caudal brainstem on spinal cord norepinephrine content and morphine-induced antinociception. Ordinate: analgesia index, Abscissa: percent reduction of norepinephrine content in the caudal half of the spinal cord. Spearman Rank Correlation Coefficient=0.05; ($p>0.05$). ●, electrolytic lesions of the raphe nuclei (n=7); ○, glutamate lesions of the raphe nuclei (n=4); ■, electrolytic lesions of the paramedian nucleus (n=3); □, glutamate lesions of the paramedian nucleus (n=2).

lation [52]. In addition, Yaksh and coworkers [70] reported shorter hot plate and tail flick latencies following raphe magnus lesions, although the differences were not statistically significant. The results of the present experiments confirm our previous report and extent these observations to include hyperalgesia following raphe pallidus lesions. These findings lead to the suggestion that the caudal raphe nuclei (n. raphe magnus and n. raphe pallidus) exert a tonic inhibition of spinal cord neuronal systems involved in the transmission of nociceptive information. Such a suggestion is consistent with the growing body of evidence that activation of bulbospinal 5-HT systems results in inhibiton of pain-transmission neurons in the spinal cord dorsal horn [3, 6, 22, 23, 26, 40, 68]. Iontophoretically applied 5-HT has also been reported to inhibit pain-responsive dorsal horn interneurons [8,53]. In addition, there is evidence which supports a presynaptic inhibitory action on primary afferent transmission exerted by descending 5-HT systems [37, 50, 51]. Furthermore, direct evidence for the regulation of nociceptive threshold by serotonergic systems has been provided by the demonstration that direct application of 5-HT into the spinal cord subarachnoid space produces a dose-dependent decrease in responsiveness to noxious peripheral stimulation [72].

We have previously reported that lesions of the nucleus raphe magnus result in attenuation of the capacity of morphine to induce antinociception [52]. More recently, others have confirmed these results [13,70]. These findings lead to the suggestion that the bulbospinal 5-HT system orginating in the nucleus raphe magnus is an important constituent of the neuronal assemblage which mediates morphine-induced analgesia. However, the results of these studies do not unequivocally implicate 5-HT-containing cells, since the possibility exists that the lesion-induced effects resulted from damage to structures or fiber systems adjacent to the raphe magnus. Thus, a major problem with these studies is the absence of adequate delimitation of the anatomical site responsible for the lesion effects. In the present study, the following information was obtained to more precisely define the role of 5-HT-containing cells in the raphe nuclei in mediating morphine-induced analgesia and pain perception:

(1) Evidence that damage to a particular site results in a given effect can be obtained by correlating the degree of damage to the suspected anatomical site with the degree to which the effect is altered by the damage. Thus, if the raphe nuclei are necessary for the expression of morphine-induced analgesia, then a positive correlation should exist between

the extent of raphe destruction and the degree of attenuation in the capacity of morphine to induce analgesia. The results of these studies indicated such a correlation does indeed exist for both electrolytic and glutamate lesions of the raphe magnus and the raphe pallidus. Furthermore, destruction of approximately 30% or more of either nucleus resulted in nearly complete blockade of the antinociception resulting from 5 mg/kg of morphine.

(2) Lesions of the raphe nuclei were produced by the injection of microliter quantities of glutamic acid in an attempt to selectively destroy neuronal perikarya while minimizing damage to surrounding structures such as blood vessels, glia, and fibers passing near the raphe area. The selectivity of glutamate-induced lesions has been investigated in light- [58] and electron-microscopic preparations [46,64]. Light-microscopic examination of glutamate-induced lesions in the rostral hypothalamus stained for degenerating fibers using the Fink-Heimer technique revealed no more damage to axons of passage than that which occurs following insertion of the injection cannula alone [57]. Electron-microscopic examination of cortical sites following iontophoretic application of glutamate revealed acute swelling of neural elements (somata, dendrites and axons) in the area of the injection [64]. However, these changes were reversible within several days following the injection with the exception of neuronal perikarya which became electron opaque and shrunken. Similar studies of sites in the rat striatum 21 days following glutamate injection indicated that all intrinsic striatal neurons had degenerated, but bundles of axons passing through the area of injection were intact and appeared normal [46].

In addition, the functional integrity of axons passing through the area of damage induced by glutamate injection is suggested by the persistence of flash-evoked potentials following the injection of glutamate into the optic tract [57]. Similarly, the neurochemical integrity of small diameter noradrenergic axons passing through glutamate injection sites has also been reported [27]. These studies showed that injections of glutamate into the ventral parabrachial nucleus, an area that includes dense projections to and from the nucleus locus coeruleus, failed to alter NE content in the cortex, spinal cord, or medial brain stem.

Additional support for the selectivity of glutamate is provided by numerous studies of the neurotoxic properties of kainic acid, a rigid analog of glutamate, which exhibits qualitatively similar, but more potent neurotoxicity. For example, electron-microscopic examination of the damage resulting from local injections of kainic acid into the rat striatum has revealed destruction of local neurons and their axons, but no damage to extrinsic axons [16, 46, 54].

Although it is not possible at the present time to make a definitive statement concerning the axon-sparing properties of glutamate and its neurotoxic analogs in every area of the central nervous system, it may be suggested that the damage to axons of passage is certainly less than that produced by electrolytic lesioning methods. Thus, it may be concluded from the present studies that since both glutamate and electrolytic lesions were equally effective in attenuating the capacity of morphine to produce analgesia, it is unlikely that damage to fibers passing near the raphe area was responsible for the lesion-induced effects. Therefore, the lesion-induced attenuation of morphine analgesia most likely resulted from destruction of neuronal perikarya residing in the raphe area.

(3) The hypothesis that destruction of 5-HT-containing neurons in the caudal brain stem raphe nuclei is responsible for lesion-induced alterations in morphine analgesia was tested by correlating changes in analgesia with changes in spinal cord 5-HT content resulting from raphe lesions. The degree of damage to the caudal raphe nuclei (magnus and pallidus) produced either electrolytically or by the local injection of glutamic acid was found to vary (Figs. 1 and 4). Thus, such lesions should reduce spinal cord 5-HT content to varying degrees. Furthermore, if morphine analgesia is dependent on the integrity of bulbospinal 5-HT pathways, then lesion-induced alterations in spinal cord 5-HT content should be correlated with changes in the capacity of morphine to induce analgesia. Such a correlation was demonstrated (Fig. 5) which indicates that the expression of morphine analgesia is mediated, at least in part, by the descending 5-HT pathways originating in the 5-HT-containing raphe nuclei of the caudal medulla. These conclusions are consistent with reports demonstrating attenuation of morphine analgesia following neurotoxin-induced reduction of spinal cord 5-HT resulting from intraspinal administration of 5,7-dihydroxytryptamine [21] or intraventricular 5,6-dihydroxytryptamine [65].

(4) The possibility exists that damage to bulbospinal NE fibers which pass near the raphe area was responsible for the observed lesion-induced effects. This possibility was examined by correlating alterations in morphine analgesia with changes in spinal cord NE content. Lesions were placed in various midline sites such as the raphe magnus, raphe pallidus, and medial brain stem sites; the paramedian reticular nucleus. If these medial NE fibers are involved in the mediation of morphine analgesia, then a high degree of association should exist between lesion-induced changes in spinal cord NE content and morphine analgesia. However, no statistically significant association between these two variables could be demonstrated. This analysis leads to the conclusion that damage to bulbospinal NE axons passing near the midline raphe nuclei does not contribute to the attenuation of morphine analgesia following raphe lesions.

An unexpected result of these studies was the observation that animals with lesions of the nucleus reticularis paramedianus exhibited an attenuated response to morphine. Since the major projections of the paramedian reticular nucleus are to the fastigial nuclei of the cerebellum [11], these data suggest the possible involvement of the deep cerebellar nuclei in the mediation of morphine analgesia. In addition, the fastigial nuclei project to the raphe magnus and may be important in controlling raphe activity. The role of these nuclei in modulating reactivity to noxious stimuli is supported by preliminary data from this laboratory indicating that lesions of the fastigial nuclei attenuate morphine analgesia while electrical stimulation of this region produces antinociception (Proudfit, unpublished observations).

In summary, these studies have demonstrated that lesions of the bulbospinal 5-HT nuclei, raphe magnus and raphe pallidus, produce decreased nociceptive threshold and attenuation of morphine-induced analgesia. Furthermore, the significant correlation between lesion-induced changes in spinal cord 5-HT content and morphine analgesia lends support to the conclusion that the descending 5-HT systems are necessary for the mediation of morphine effects. Finally, the effects of midline lesions in the caudal brain stem do not appear to require destruction of bulbospinal NE systems descending near the raphe nuclei.

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