Descending Inhibitory Influences From Periaqueductal Gray, Nucleus Raphe Magnus, and Adjacent Reticular Formation. I. Effects on Lumbar Spinal Cord Nociceptive and Nonnociceptive Neurons

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SUMMARY AND CONCLUSIONS

1. This study examined the inhibitory effects of conditioning stimuli delivered to the periaqueductal gray (PAG), nucleus cuneiformis (CU), nucleus raphe magnus (NRM), nucleus reticularis gigantocellularis (NGC), and nucleus reticularis magnocellularis (NMC) on functionally identified neurons of the lumbar spinal cord dorsal horn in chloralose-anesthetized or decerebrate cats.

2. Neurons were classified according to their responses to a variety of cutaneous stimuli as low-threshold mechanoreceptive (LTM), wide dynamic range (WDR), or nociceptive specific (NS). The major aim of this study was to determine whether there was a difference in the effectiveness of brain stem stimulation-produced inhibition of nociceptive (noci) neurons (consisting of both WDR and NS neurons) and the LTM nonnociceptive (nonnoci) neurons. There were no statistical differences in the susceptibility of WDR and NS neurons to brain stem-induced inhibition.

3. Most neurons tested could be inhibited by stimulation of any of the brain stem regions tested. In all cases the percentage of noci neurons inhibited from a given region was higher than the percentage of nonnoci neurons; however, this difference was only statistically significant in the case of NMC stimulation.

4. Threshold current intensities necessary to produce inhibition were determined for each neuron from each stimulation site. Although there was a trend for noci neurons to require slightly lower current intensities, there was in fact no statistically significant difference in the inhibitory thresholds between noci and nonnoci neurons for any of the regions tested.

5. A comparison of the mean threshold currents for the five regions studied revealed that the lowest stimulation currents were obtained in NMC with NRM, CU, NGC, and PAG, each requiring progressively higher current intensities in order to produce inhibition.

6. These results indicate that stimulation in PAG and NRM not only inhibits the responses of noci neurons but also those of nonnoci neurons. Moreover, stimulation in reticular regions adjacent to these two regions is effective in inhibiting the responses of both noci and nonnoci neurons.

INTRODUCTION

There has been a great deal of interest in recent years in the effects of stimulating various brain stem regions on the responses of somatosensory neurons. A major impetus for these studies was the finding that stimulation in the periaqueductal gray matter (PAG) could produce analgesia (24, 38, 40, 45–47, 53, 54). The analgesia obtained by stimulation in the PAG is presumed to result from the activation of a descending inhibitory pathway relaying in nucleus raphe magnus.
(NRM) (7, 49, 57) and terminating in the dorsal horn (5, 6, 24, 41). Consistent with this hypothesis, a number of studies have shown that stimulation in these two regions selectively inhibits nociceptive (noci) neurons in the spinal dorsal horn (8, 20, 25, 32, 36, 45). However, a number of recent studies have clearly demonstrated that nonnociceptive (nonnoci) neurons can also be inhibited by stimulation in PAG or NRM (11, 17, 43, 48, 61). Moreover, stimulation in reticular regions adjacent to the PAG and NRM has also been found to be effective in inhibiting spinal cord dorsal horn neurons (11, 13, 28, 34, 43, 61, 63). The relative degree of inhibition of noci and nonnoci neurons resulting from stimulation in the PAG, NRM, and adjacent reticular areas has not been clearly established.

The experiments described here were performed in order to examine whether stimulation in the PAG and NRM preferentially inhibits noci neurons and to what extent stimulation of sites in adjacent reticular formation produces similar effects. In these studies the effects of stimulation in various regions of the brain stem were examined on both noci and nonnoci neurons in the spinal cord dorsal horn. The noci neurons consisted of nociceptive-specific (NS) and wide dynamic range (WDR) neurons (50). WDR neurons, although responding also to nonnociceptive afferent input, have been included in the noci group because they have been strongly implicated in the relay of pain-related signals to higher levels of the nervous system (51). The use of multiple stimulation sites within the same animal allowed for a direct comparison of the relative effectiveness of the various regions stimulated in inhibiting the responses of individual neurons. Some results from these studies have been reported previously (30). The following paper (19) describes similar studies on the effects of brain stem stimulation on the responses of medullary dorsal horn neurons.

METHODS

Experiments were performed on chloralose-anesthetized or decerebrate cats weighing between 2.5 and 4 kg. In the former, following induction with an intrathoracic injection of sodium pentothal (25 mg/kg), the left femoral vein and artery were cannulated for the administration of chloralose (60 mg/kg) and Flaxedil (gallamine triethiodide) and for monitoring arterial blood pressure, respectively. Adequate anesthesia was ensured throughout the course of the experiments by maintaining a level where no blood pressure changes, pupillary dilatation, or withdrawal reflexes were evoked by a nociceptive stimulus. In decerebrate animals, after induction and when the cat was deeply anesthetized, an array of four monopolar stimulating electrodes was stereotaxically lowered through the rostral brain stem (AP+2.0) and electrolytic destruction of the tissue at 1.5-mm intervals during three successive descents of the array along the coronal plane were made. Decerebration was verified histologically to be complete in all cats. A tracheostomy was performed to allow for artificial ventilation. End-tidal CO₂ and blood pressure were monitored and maintained within physiological limits (3.5-4.5% and 80-140 mm Hg, respectively). Body temperature was maintained at 37.5°C with a thermostatically controlled heating pad. The hindpaw was closely shaved to enable localization of the receptive fields. The animal was then placed in a stereotaxic frame and the spinous process of a lumbar vertebra clamped to improve stabilization. Craniotomies were performed to allow the introduction of the stimulating electrodes; the exposed cortical surfaces were subsequently covered with agar.

Bipolar concentric stainless steel electrodes (Rhodes NE-100; tip separation, 1.0 mm; outer diameter, 0.5 mm; inner diameter, 0.2 mm) were stereotaxically lowered to the mesencephalic and medullary regions (the latter at an angle of 20° to the vertical). These electrodes were mounted as arrays on two electrode carriers. The medial electrode of the mesencephalic pair was aimed at the PAG (AP+0.6, H0.0, L1.5) and the lateral electrode was aimed at nucleus cuneiformis (CU) (AP+0.6, H1.5, L2.5) as described by Taber (60) (labeled central tegmental field in Berman (9)). The medial electrode of the medullary pair was aimed at NRM (AP-7.0, H-9.0, L0.0) and the lateral electrode at NGC (AP-7.0, H-7.5, L1.5) or NMC (AP-7.0, H-9.0, L1.5), depending on the array used. All nonmidline stimulation sites were ipsilateral to the recording site in the spinal cord (right side). All sites were subsequently verified by histological means. Stimulation sites were marked on diagrams of the appropriate AP level of the brain stem. For sites near the border of PAG with CU, it was easy to determine on which side of the border they were because of the very clear cytoarchitectonic boundary between these two regions. In a few cases the PAG electrode partially penetrated the aqueduct or was in the dorsal midline region (PAG dorsalis; see Ref. 35). Data ob-
tained from these sites have not been included in the analysis. For sites in the caudal brain stem it was sometimes more difficult to decide whether a given site was in or out of NRM or in NMC or NGC. Sites located just medial to the facial nucleus or just dorsal to the pyramidal tract have not been included in the data since it was judged that they were in nucleus reticularis paragiganto-cellularis (1). For this reason we have provided detailed reconstructions of the stimulation sites showing the location of the stimulation sites and the approximate boundaries of the regions. For analysis purposes stimulation sites were classified according to the location of the points in these sections (Fig. 1); for example, all the points falling within the boundary indicated for NGC were classified as being in NGC.

A laminectomy was performed exposing the lumbar-sacral enlargement and in later experiments also the region of C7. After opening the dura mater and in some cases dissecting away the pia mater, warm mineral oil or 2.5% agar was used to cover the spinal cord. Depending on the degree of respiratory movement, a pneumothorax and/or occasionally a modified pressor foot (18) were employed to help stabilize the spinal cord. Local temperature in the area was monitored with a thermistor and maintained at 35–37°C via the use of heating lamps. Occasionally the steroid dexamethasone (Dexamone 2, iv, 0.5 ml/kg) was used to help prevent edema of the spinal cord. Also an infusion of lactated Ringer solution or 6% dextran (3.3 ml/h) was sometimes employed to help maintain blood pressure.

Single-unit extracellular recordings were made from the lumbar spinal cord dorsal horn with carbon-fiber microelectrodes (4) or glass-coated, platinum-plated, tungsten microelectrodes (7) with resistances in the 1- to 3-MΩ range. The recording electrode was gradually lowered through the spinal cord dorsal horn with a microdrive while monitoring neuronal activity evoked by mechanical stimulation of the hindpaw. The responses of the neuron to brushing the skin (nonnoxious stimulation) and pinching the skin with serrated forceps (noxious stimulation) were examined. A noxious thermal stimulus (heat lamp) was routinely employed to aid in characterizing the neurons. The intensity, distance, and duration used were those that produced a painful sensation when tested on the investigator. Most neurons that responded to noxious mechanical stimuli also responded to noxious thermal stimuli. When a responding neuron was found, the receptive field was mapped and the cells categorized, in accordance with previous work (50), as 1) low-threshold mechanoreceptive thermal (LTM) for those neurons responding only to nonnoxious tactile stimuli such as brushing hairs or light touch; 2) wide dynamic range (WDR) for those neurons responding to low-threshold mechanoreceptive and with an increased discharge to noxious stimuli such as pinching, pin pricking, and noxious heating; and 3) nociceptive specific (NS) for those neurons responding only to noxious stimulation. In some experiments a microelectrode was inserted into the ipsilateral lateral cervical nucleus at the C2 level (0.5–1.0 mm below the surface and 0.0–0.5 mm lateral to root entry) and was used to identify, by antidromic stimulation, lumbar dorsal horn neurons projecting to that region. The electrode was placed at a site where neurons having large biphasic action potentials and receptive fields on the hindlimb and sometimes also on the forelimb could be recorded. The antidromic nature of the response was based on three criteria: 1) ability to follow 300- to 500-Hz stimulation at C1, 2) ability to follow paired pulses, 3) constant latency, and 4) collision at the appropriate critical interval with the spontaneous or evoked activity (26). Only well-isolated units were used and their activity was continuously monitored on a digital oscilloscope. The trace was triggered from a window discriminator and the signal delayed (Neurolog NL 740 analog delay) to allow the whole waveform of the action potential including the pretriggered segment to be observed at a sweep speed of 0.2–0.5 ms/division. In addition, a “control” action potential was usually stored and succeeding ones superimposed so that they could be compared to ensure that the same unit was being evoked by electrical or natural stimuli from all regions of the receptive field. After characterization, the cell was orthodromically excited by an electrical stimulus applied by percutaneous needle electrodes placed in the center of the cell’s receptive field, or, in some cases, by a gentle mechanical stimulus applied either by an electromechanical transducer or by a solenoid-controlled air jet. The latter two forms of excitation were nonnociceptive in character and were used to drive both low-threshold mechanoreceptive and wide dynamic range neurons.

In order to minimize differences in afferent drive and to enable an accurate determination of inhibitory threshold, each neuron was excited at just-suprathreshold intensity so as to produce consistently just one or two action potentials per stimulus. During testing (see below), this response was carefully monitored and the peripheral stimulus adjusted, if necessary, to maintain a constant level of excitation. This use of just-suprathreshold electrical excitation most probably activates the larger diameter afferent fibers. Thus it should be noted that descending inhibitory influences were assessed on the nonnociceptive afferent-evoked responses of the wide dynamic range neurons and, presumably, on the larger A-δ nociceptive affer-
FIG. 1. Reconstruction of all the stimulation sites used in this study. The location of each symbol marks the site of stimulation and its shape indicates the average current threshold needed to inhibit the noci cells in that particular experiment (symbols that are enclosed in a circle are from cats in which only nonnoci cells were encountered and reflect the mean for those cells). Insert gives values for symbols in microamperes. Abbreviations: AQ, cerebral aqueduct; CU, nucleus cuneiformis; DR, dorsal raphe; IO, inferior olive; NGC, nucleus reticularis gigantocellularis; NMC, nucleus reticularis magnocellularis; NRM, nucleus raphe magnus; PAG, periaqueductal gray; PT, pyramidal tract; SO, superior olive; VII, facial nucleus; 4, trochlear nucleus.

Ent-evoked responses of nociceptive-specific neurons (50).

The conditioning stimulus consisted of a 500-Hz, 100-ms train of 0.1-ms pulses (400 µA maximum) delivered 130 ms prior to the peripheral test stimulus (i.e., the test stimulus occurred 30 ms after the end of the conditioning train). Previous work in our laboratory and by others (56, 63; unpublished observations) has shown that this conditioning-test interval is in the optimal range for assessing inhibition, since the time course for inhibition is maximal at this point. Conditioning current intensities were determined by measuring, with a differential amplifier, the voltage drop across a 1,000-Ω resistor connected in series between the stimulus isolation unit and the electrode. The intensity of the conditioning current was varied until a minimum current was found that blocked the neuron’s response in at least three consecutive conditioning trials, each of which was preceded by at least one control trial. This value was referred to as the inhibitory threshold and was the parameter used to compare the degree of selectivity of the inhibition from each brain stem region and to determine the relative effectiveness of the various regions in producing the observed effects.

After the completion of data collection, lesions were made at the stimulation sites (30 µA anodal, 30 s) and the animal then perfused with 0.9% saline followed by 10% buffered Formalin containing 1% potassium ferrocyanide. The potassium ferrocyanide reacts with the iron deposited by the stimulating electrode to produce a highly visible
blue stain (Prussian blue reaction). The brain was then left in 10% buffered Formalin and subsequently sectioned at 90 µm, mounted, and counterstained with cresyl violet.

A variety of statistical tests have been employed to analyze the data in this study. The difference of proportions and the difference of means have both been used to show differences based on the pooling of all the data points in the study. For more refined analysis, the weighted paired t test and the Wilcoxon paired sample tests were employed. While the weighted paired t test allowed for the elimination of any problems associated with pooling of the experiments, the Wilcoxon paired sample test allowed for the elimination of differences that may have been encountered during the course of the experiment, i.e., slight variations in the level of anesthesia. In almost all cases all the tests yielded the same result. All statistical tests were two tailed at the 5% significance level. References to significance relate to the means test unless otherwise stated.

RESULTS

The effects of brain stem stimulation were studied on 115 nonnoci and 138 noci neurons (109 WDR and 29 NS) in the spinal cord dorsal horn of 43 cats (4 decerebrate). On the basis of micromanipulator readings and functional characteristics, these neurons were thought to be located in laminae IV, V,
and VI region of the spinal cord. Twenty-nine neurons (24 LTM, 5 WDR) were antidromically activated from the ipsilateral lateral cervical nucleus and were presumed to be spinocervical tract neurons. The results obtained from decerebrate cats did not differ from those obtained in anesthetized cats and the results from projection neurons did not differ from those of nonprojection neurons, and thus they have all been pooled together. The stimulation sites were histologically verified to be in the PAG, CU, NRM, NGC, and NMC in 15, 16, 21, 20, and 17 instances, respectively, and are shown in Fig. 1. Stimulation of all these regions was found to be effective in inhibiting the sensory responses of both nonnoc and noc neurons. Figure 2 provides an example of typical records showing the inhibition of a just-suprathreshold response of a nonnoc neuron by stimulation in CU, NRM, and NGC.

Brain stem stimulation caused facilitation rather than inhibition of the response in three cells (one nonnoc and two noc's). The regions producing this effect were NRM in three of the cases and PAG in the case of one of the noc's. It is possible that other neurons were excited at a short latency but this was obscured by the artifact of the conditioning train.

In terms of incidence of inhibition, NMC (86%), NRM (84%), CU (82%), and NGC (79%) were all similar in effectiveness. Compared to these, PAG (66%) was found to be the least effective. This pattern was the same for noc and nonnoc neurons and similar within the noc group for WDR and NS neurons (Table 1). In all cases the percentage of noc neurons inhibited from a given region was higher than the percentage of nonnoc neurons; however, this difference was only statistically significant in the case of NMC stimulation. It should be kept in mind that those cells that were unaffected may have been inhibited by stimulation currents in excess of our maximum of 400 μA. There were no significant differences in the incidence of inhibition of WDR and NS neurons.

The threshold brain stem stimulation current depended markedly on the level of the peripheral stimulus. Increasing the peripheral stimulation intensity easily overcame the block produced by the conditioning train, although a partial inhibition could still be seen and was manifested by an increased latency of the response and/or a decreased number of spikes in the discharge. This could be readily observed by comparing poststimulus histograms of 10 control and 10 conditioned trials. More than 90% of the data were collected on responses evoked by electrical skin stimulation but some cells (both LTM and WDR) were tested by using an air jet or an electromechanical transducer to evoke the response, and no differences in susceptibility of the responses to inhibition were noted.

**Analysis of current thresholds**

The main aim of this study was to determine the relative inhibitory effectiveness of the various brain stem regions and to see whether any of them inhibited preferentially noc or noc neurons. Since all regions inhibited a high percentage of all the units tested, the analysis concentrated on comparing inhibitory thresholds. Table 1 gives the mean current thresholds necessary for inhibiting nonnoc and noc cells. Except for CU there was no statistical difference between the currents necessary to inhibit WDR and NS neurons.

### Table 1. *Relative effectiveness of regions*

<table>
<thead>
<tr>
<th>Region</th>
<th>Type</th>
<th>Total No.</th>
<th>Percent</th>
<th>Current, μA</th>
</tr>
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<tbody>
<tr>
<td>PAG</td>
<td>nonnoc</td>
<td>31</td>
<td>17</td>
<td>55</td>
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<tr>
<td></td>
<td>noc</td>
<td>49</td>
<td>36</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>WDR</td>
<td>35</td>
<td>26</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>14</td>
<td>10</td>
<td>71</td>
</tr>
<tr>
<td>CU</td>
<td>nonnoc</td>
<td>39</td>
<td>30</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>noc</td>
<td>58</td>
<td>50</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>WDR</td>
<td>46</td>
<td>41</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>12</td>
<td>9</td>
<td>75</td>
</tr>
<tr>
<td>NRM</td>
<td>nonnoc</td>
<td>56</td>
<td>43</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>noc</td>
<td>68</td>
<td>61</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>WDR</td>
<td>52</td>
<td>46</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>16</td>
<td>15</td>
<td>94</td>
</tr>
<tr>
<td>NGC</td>
<td>nonnoc</td>
<td>60</td>
<td>45</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>noc</td>
<td>59</td>
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<td>83</td>
</tr>
<tr>
<td></td>
<td>WDR</td>
<td>50</td>
<td>43</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>9</td>
<td>6</td>
<td>67</td>
</tr>
<tr>
<td>NMC</td>
<td>nonnoc</td>
<td>46</td>
<td>36</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>noc</td>
<td>60</td>
<td>55</td>
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<tr>
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<td>WDR</td>
<td>44</td>
<td>41</td>
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<tr>
<td></td>
<td>NS</td>
<td>16</td>
<td>14</td>
<td>88</td>
</tr>
</tbody>
</table>

Values for current are means ± SE.
and, therefore, the two groups have not been separated in the following analysis. These data reveal that within the caudal brain stem, the highest thresholds were in NGC. NGC thresholds were comparable to the CU thresholds and lower than PAG thresholds. Within each region except NGC and NMC,
there is a consistent trend for the mean current threshold necessary to inhibit the noci cells to be slightly lower than that for the nonnoci cells; however, in no case was this difference statistically significant. Reference to Fig. 1 allows for an inspection of the relative degree of effectiveness of each stimulation site by showing the average current necessary to inhibit all the noci cells or, in a few cases when no noci cells were recorded, the average for the nonnoci neurons in each cat. Clustering of particularly effective sites (i.e., low threshold) around a specific subregion was not apparent. The distributions of current thresholds, shown in Fig. 3, indicate that very few cells required currents close to our maximum cutoff of 400 μA, and thus it is unlikely that raising the maximum by a few hundred microamperes would have altered the results significantly. In addition, within each region studied there is a large overlap between the distributions of currents necessary to inhibit the nonnoci and noci cells. In order to confirm these findings, which are suggestive of a lack of preferential inhibition, the data were analyzed in more detail, as described below.

**Comparison of noci versus nonnoci currents**

In order to reduce possible errors produced by averaging data from different animals where electrode placements, anesthetic levels, and physiological conditions unavoidably vary, the following analysis avoided pooling interanimal results. Figure 4A illustrates the relative effectiveness of PAG and CU

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**Fig. 5.** Reconstruction of the stimulation sites where results from both noci and nonnoci cells were obtained in the same experiment. Symbols mark the stimulation sites and their shape represents the ratio of average current intensity necessary to inhibit the nonnoci cells divided by the average current intensity necessary to inhibit the noci cells. Insert gives the range of ratios for each symbol.
with respect to noci and nonnoci cells. Each point represents the pooled data from all neurons in one experiment. The coordinates \((x, y)\) are the average current intensities required to inhibit noci cells and nonnoci cells, respectively. The 45° line \((x = y)\) on these graphs indicates where points should lie if the thresholds for inhibition were identical. The points are scattered on either side of the line for both PAG and CU, suggesting that there was no specificity. A statistical analysis (weighted paired t test) verified that there was no significant difference in the current thresholds required to inhibit noci cells and nonnoci cells. Similar results were obtained for each of the regions studied in the caudal brain stem, as shown in Fig. 4B.

Figure 5 shows the sites where the electrodes were correctly positioned in cats where brain stem effects were studied on both nonnoci and noci cells. Each symbol represents the ratio of the average current intensity necessary to inhibit the nonnoci cells divided by the average intensity necessary to inhibit the noci cells by stimulation at that site. This presentation of the data was used to facilitate the visualization of possible subregions where stimulation may have been selective for inhibiting noci cells. It can be seen that within any one region there is no consistent clustering of points representing ratios greater than 1.5. (However, many of the stimulation sites in rostra1 medulla and rostra1 midbrain had ratios greater than 1.1, suggesting a preferential effect on noci neurons from those regions.) Thus it appears that when comparing the individual regions and their effects on nonnoci and noci cells, neither percentages of cells inhibited nor the average current intensities provide evidence in support of a selective inhibition of one class of cell.

Comparison of effectiveness of five regions

It is interesting to look at the plots of current intensities in terms of current necessary to inhibit the same cell from two different regions in the same animal. Figures 6 and 7 compare the effectiveness of the various areas; WDR, filled circles; and NS, filled triangles. Each point represents thresholds for inhibiting the same cell from two different regions. Regions compared are A, NRM versus NGC; B, NRM versus NMC; and C, NMC versus NGC.
FIG. 7. Plots comparing the relative effectiveness of stimulation of regions within the midbrain to those within the caudal brain stem. Each symbol represents a particular type of neuron as follows: LTM, open circles; WDR, filled circles; and NS, filled triangles. Each point represents thresholds for inhibiting the same cell from two different regions. Regions compared are A, PAG versus NRM; B, CU versus NRM; C, PAG versus NGC; D, CU versus NGC; E, PAG versus NMC, and F, CU versus NMC.
against one another. This analysis, as applied to the caudal brain stem, is shown in Fig. 6 and reveals the relative effectiveness of NRM, NMC, and NGC. The effectiveness of NRM versus NGC, Fig. 6A, shows that a majority of the cells fall below the 45° line, suggesting that NRM is more effective than NGC. When comparing NRM and NMC one finds them to be quite similar in effect, as indicated by the grouping of cells around the line (Fig. 6B). Not surprisingly Fig. 6C reveals that like NRM, NMC is more effective than NGC. Thus, as revealed also by the current threshold data (Table I), there appears to be a relatively similar degree of effectiveness from NRM and NMC, with NGC being slightly less effective. In terms of the percentage of cells that are inhibited (Table 1), the order of relative effectiveness is also NMC > NRM > NGC. An analysis of the relative effectiveness of the midbrain regions to the caudal brain stem regions is illustrated in Fig. 7A-F. The effects of PAG and CU versus each of NRM, NGC, and NMC are shown adjacent to one another to allow for further comparisons. In general, the plots show that PAG is less effective than the regions in the caudal brain stem, whereas the differences between CU and the same regions are less pronounced. Though not shown, the implication that PAG is less effective than CU is borne out using such an analysis.

**DISCUSSION**

**Methodological considerations**

Most previous electrophysiological studies on the effects of NRM and PAG stimulation on spinal cord somatosensory neurons have concluded that nociceptive neurons are inhibited to a greater extent than nonnociceptive neurons (8, 10, 20, 25, 32, 36, 45). These conclusions have been based primarily on experiments that have examined the percentage of nociceptive versus nonnociceptive cells inhibited, or on the percent reduction of their evoked responses following stimulation in the brain stem. One important difference between the present study and these previous studies was our careful use of peripheral stimuli that were just suprathreshold for exciting the neurons, so that the inhibitory effects were tested on neurons having a similar degree of afferent input. However, in many cases the inhibition was also seen on a suprathreshold response as a reduction in the number of spikes and/or an increase in latency. The use of suprathreshold responses could lead to differences in the apparent susceptibility of a class of neurons to be inhibited due to differences in afferent drive. More intense stimuli, such as used in previous studies (28, 39, 55), would activate higher threshold afferents and these may be preferentially inhibited. Since the responses to these high-threshold afferents were not examined in the present study (for WDR neurons), this may account for the lack of a preferential effect on nociceptive afferent input.

The conditioning stimulus threshold is dependent not only on the intensity of afferent drive but also on the train frequency, train length, and pulse width. In order to reduce the problems associated with current spread (in terms of anatomical localization of effective sites), we employed a 500-Hz train, which gives the lowest current thresholds due to a maximum degree of temporal facilitation. Studies where low-frequency or short-duration trains were used would tend to require higher intensity currents to produce an equal inhibitory effect and, assuming a maximum current cutoff limit was employed, would reveal a smaller percentage of neurons affected by brain stem stimulation.

One other difference between the present study and previous studies is the use of current-intensity thresholds as a measure of inhibitory effectiveness. Increasing the current intensity increases the current spread and thus, the volume of tissue excited. It is clear from our unpublished observations and those of other researchers (13, 17, 28, 34, 63) that increasing the intensity of stimulation also increases the magnitude of inhibition, at least for a certain range above threshold. Our method is based on the assumption that the magnitude of inhibitory input onto a given neuron from stimulation of a given region is related to some extent to the current intensity and that a certain minimum amount of inhibition is required in order for it to be detectable. By carefully controlling the level of excitation of each neuron (i.e., response...
to peripheral stimulation), we ensured that the brain stem stimulus at threshold (current threshold) produced approximately the same degree of inhibition on the responses of each of the neurons tested. Thus the greater the inhibitory input to a cell, the lower the current threshold required to produce the just-observable inhibition.

**Brain stem effects on noci versus nonnoci neurons**

Most neurons of each group were inhibited with only small differences in the incidence of inhibition, which except for NMC were not statistically significant. When these inhibitory effects were analyzed in terms of the current threshold necessary to produce inhibition, again no significant differences were noted, even in the case of NMC. If the responses of nonnoci neurons were influenced by fewer descending fibers (or each descending fiber had a weaker inhibitory effect) compared with those of noci neurons, then one would expect that higher currents would be required in order to produce an observable inhibition of nonnoci neuron responses.

One possible reason for the apparent preferential inhibition of noci neurons by PAG and NRM stimulation, as found in some of the other studies, was their use of supra-threshold peripheral stimuli. With supra-threshold stimuli, nonnoci neurons tend to be driven more strongly, synchronously, and for a shorter duration than noci neurons (64). Thus it would be more difficult to see an inhibitory effect on the nonnociceptive response than on the noci response.

This study revealed that LTM, WDR, and NS neurons were equally susceptible to descending influences when activated to comparable levels. Whether the observed inhibition was mediated by presynaptic and/or postsynaptic mechanisms cannot be determined, though other work has shown both to be present (see discussion, Ref. 19). It should be stressed that the present study assessed the inhibition primarily on the nonnociceptive responses of WDR neurons. Due to the different response characteristics of the WDR neurons to nonnoxious and noxious stimuli (64), it is likely that the same inhibitory input, if postsynaptic, would be more effective in reducing the transmission of nociceptive information than nonnociceptive information. Moreover, there may be a preferential presynaptic inhibition of C-fiber input. It should be noted, however, that a reduction in the transmission of information may be seen as a change in the encoding properties of a neuron in response to a given input (i.e., a change in the interspike interval) rather than simply a reduction in the absolute number of spikes. To date this sort of change in response resulting from descending systems has not been thoroughly investigated. Such a loss, which is more subtle and thus harder to observe, may also account for the lack of behavioral evidence showing any tactile deficits since the tests employed may not be sensitive enough (42, 47, 58).

Although some previous studies on PAG-induced inhibition of spinal cord sensory neurons have reported that no or very few nonnociceptive neurons were inhibited (8, 36, 45), other studies have described PAG-induced inhibition of both noci and nonnociceptive neurons (11, 20, 61). Previous studies on NRM-induced inhibition have also yielded conflicting results. In agreement with the present study, a number of studies (43, 61) have found that nonnociceptive cells were inhibited as well as noci cells, while other studies (20, 25, 32) reported that primarily noci cells were inhibited. Studies that have looked at the effects of stimulation of the NGC, NMC, and CU and their ability to inhibit noci and nonnociceptive cells in the spinal cord dorsal horn (11, 13, 43, 61) have reported, in agreement with our findings, that both classes can be inhibited. It should be pointed out that this study has focused on the relative degree of inhibition of noci and nonnociceptive neurons and should not be confused with studies that have investigated whether the nociceptive- or the nonnociceptive-evoked response of individual WDR neurons is specifically affected (28, 39, 55).

**Origin of descending influences**

The various methods used in this study have revealed a remarkable similarity in the inhibitory effects produced by stimulation in PAG, CU, NRM, NGC, and NMC. Stimulation in the caudal brain stem and CU produced similar results. Stimulation in the PAG was found to be less effective in producing inhibition, both in terms of the percentage of neurons inhibited and in terms of
the average threshold current necessary to inhibit the neurons. The current-threshold data suggest that the volume of tissue that must be activated to produce a noticeable effect is larger in PAG than in the other regions. This difference may be due to a low-density projection from PAG to the medullary (NRM) neurons that ultimately mediate the inhibitory effects in the spinal cord. Thus the number of neurons activated in the final inhibitory pathway to the cord may be the same from both sites.

This study allowed a comparison to be made within the same animal of the effects of stimulation of a number of regions on the same neuron. This type of analysis clearly showed that many noci and nonnoci neurons were more effectively inhibited from NMC than NRM and from CU rather than PAG. These findings rule out the possibility that the inhibitory effects resulting from stimulation in NMC or CU were due to current spread to NRM or PAG. The average current intensities found effective in this study were approximately 150 $\mu$A (0.1-ms pulse width), which would be capable of exciting the largest myelinated fibers at distances up to 700 $\mu$m (52). However, it is likely that the inhibitory effects were the result of excitation of neuronal cell bodies and/or small axons rather than large myelinated fibers and, since these are less excitable (52), a smaller volume of tissue would be involved. Thus it is clear that the effects observed could not have been due to current spread to a few circumscribed regions but rather that stimulation of a fairly wide region of the brain stem leads to inhibition of spinal cord dorsal horn neurons.

With a closer inspection of the results obtained from the regions in the caudal brain stem one finds greater similarity between NRM and NMC than either has with NGC. This finding is consistent with the well-studied anatomical pathway originating in this region and descending in the dorsolateral funiculus (5, 6, 62). These findings, together with those showing that these two regions receive inputs from both PAG and CU (1, 27), appear to support the idea of the whole region (NRM and NMC) being a functional unit (62; however see Ref. 1). Such an idea correlates well with studies on spinal reflexes where the effective regions for inhibiting flexor reflex afferents or withdrawal reflexes were those giving rise to the dorsal reticulo-spinal tract (21, 22, 65), i.e., in primarily NRM and NMC. However, pharmacological studies dealing with the microinjection of opiates into these two regions report conflicting results. One group (16) reports analgesia following microinjection into NRM but not into the adjacent NMC, while the other group (3) reports NMC to be the effective locus and not NRM. Thus although the concept of a functional unit is appealing, more work needs to be done.

NGC would appear to be different from the other two regions in the caudal brain stem in a few respects and thus may account for the results obtained. First, the midbrain regions PAG and CU project only sparsely to NGC (1, 27). Second, unlike NRM or NMC, NGC receives a strong input from spinal projection neurons (1). Third, the descending projection of NGC traverses the ventrolateral funiculus of the spinal cord, terminating in the intermediate rather than the dorsal portion of the spinal gray (5). The inhibition produced from stimulation of NGC could result from direct activation of reticulo-spinal pathways originating in the structure stimulated (5, 6) via a relay through other descending pathways, such as raphe spinal (5), or by antidromic activation of spinoreticular neurons or collaterals of spinothalamic neurons (1, 29).

The relatively lower degree of effectiveness observed when stimulating PAG compared to CU may reflect a lower density of neurons giving rise to descending inhibition, since many of the PAG stimulation sites used in the present study were in nucleus lateralis of the PAG (35). This region is different from nucleus medialis, which surrounds the cerebral aqueduct and extends also ventrolaterally in the PAG, the latter part of which has been shown to be an effective locus for stimulation-produced analgesia and the inhibition of spinal neurons (40, 45). Possibly if there were more stimulation sites placed ventrolaterally in the PAG, a better overall effect might have been produced. Alternatively, CU may simply be more effective due to the inherent characteristics of the surrounding neuropil. Such an idea is supported by the studies of Carstens et al. (11, 13) who have shown that stimulation of the PAG can suppress activity without changing the firing
threshold of the neurons, whereas stimulation of CU can suppress both. Since all the results in the present study were based on a just-suprathreshold level of excitation, any slight change in threshold could easily result in a blocked response. Thus, there is a possibility that some of the effects of PAG stimulation resulted from current spread into CU, as is suggested by the lower mean current intensities seen at the PAG-CU border (Fig. 1) compared to the rest of the PAG. Distinction between the two areas is further demonstrated pharmacologically by PAG's dependence on serotonin and CU's lack thereof (12, 31). While the anatomical pathways involved in mediating CU-induced inhibition are not known, one possibility is via NRM or NMC, since CU has been shown to project to these regions (1, 21). Another possibility is that the inhibition is mediated via locus ceruleus since CU has been shown to project to locus ceruleus (21), a region that is known to project to the spinal cord (14, 15, 44) and to produce inhibition of spinal cord dorsal horn neurons (37). It is also possible that CU stimulation antidromically activated the ascending portion of bifurcating axons from locus ceruleus, the descending portion of which terminated in the spinal cord (33). This tract traverses the brain stem just ventrolateral to the PAG (59) and correlates well with low-threshold stimulation sites for producing inhibition.

Our results, together with the studies referred to above, suggest that within the brain stem there may be more than one pathway that can give rise to descending inhibition. However, the similarity in the effectiveness between the regions studied may reflect an inherent problem associated with electrical brain stimulation; namely, the nonselective nature in which it activates neurons. It is probable that for the behaving animal, particular regions or even particular neurons within a region are active only under a given set of conditions. Variation in the level of activity of a given set of neurons would confer a greater flexibility of function due to the selective recruitment of certain pathways, which might in turn lead to a selectivity in effect.

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REFERENCES

11. Carstens, E., Bihl, H., Irvine, D. R. F., AND Zimmermann, M. Descending inhibition from medial and lateral midbrain of spinal dorsal horn: neuronal responses to noxious and nonnoxious cu-