Inhibition of dorsal-horn cell responses by stimulation of the Kölliker-Fuse nucleus

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The Kölliker-Fuse nucleus (KF) in the dorsolateral pons has been shown to be the major source of catecholamine innervation of the spinal cord. This has important implications in terms of pain control mechanisms, since catecholamine-mediated mechanisms are essential for the expression of opiate and other varieties of antinociception. This study examines the effects of KF stimulation on responses of dorsal-horn cells to innocuous and noxious cutaneous stimuli in anesthetized cats. Stimulation of the KF potently inhibits the responses of dorsal-horn cells to both noxious and innocuous stimuli. The threshold for the inhibitory effect is significantly lower for responses to noxious stimuli as opposed to innocuous stimuli. The inhibitory effect is specific to the stimulus site, as evidenced by a marked decrease in the effect following small changes in the position of the stimulating electrode in the brain stem. The latency of the effects indicates a bulbospinal conduction velocity of 4 to 5 m/sec, which is much slower than usual reticulospinal effects and is consistent with a catecholamine-mediated system. The dependence of KF-spinal inhibition on intact biogenic amines was tested by depleting the animals of these amines with reserpine pretreatment. Depletion of biogenic amines resulted in a significant decrease in the KF spinal inhibitory effects, suggesting their dependence on intact noradrenergic stores. The results of these studies are consistent with the idea that the KF-spinal system plays an important noradrenergic-dependent role in the brain-stem modulation of spinal processing of noxious, potentially painful stimuli.

KEY WORDS • pain • brain-stem stimulation • spinal cord • dorsal horn • Kölliker-Fuse nucleus
ventricle ventrolaterally along the medial border of the brachium conjunctivum and blend with the cells of the KF. The KF is found at the most ventral tip of the brachium conjunctivum.6,7

Materials and Methods
Thirty cats, each weighing between 2.5 and 4.0 kg and anesthetized with alpha chloralose (70 mg/kg), were used for this study. Following chloralose administration, the animals were placed in a spinal frame and the lumbar enlargements were exposed. Small occipital craniectomies were carried out to allow access for the stimulating electrodes to the brain-stem structures of interest. Exposed neural tissue was covered with warm (37°C) saline or mineral oil. Blood pressure, end-expiratory CO₂, and body temperature were monitored and maintained within normal limits.26 During recording periods the animals were paralyzed with gallamine triethiodide and mechanically ventilated. If there was pupillary dilation or increase in blood pressure during noxious stimulation of the skin, or if the animal demonstrated any withdrawal reflexes secondary to such stimulation as the paralyzing agent wore off, the animal was given a supplemental dose of chloralose (30 mg/kg).

With the aid of coordinates from a standard atlas,6 monopolar tungsten electrodes coated with Parylene were placed in the mesencephalic nucleus of the trigeminal nerve. Single unit activity from this nucleus elicited by jaw opening served to identify it and allow for correction of the KF location derived from the atlas.39,40

The electrode was then moved to the corrected target location and was used to deliver trains of stimuli to the target nucleus. The brain-stem stimuli consisted of 200 μsec biphasic pulses at 100 Hz with amplitudes ranging from 5 to 400 μA. The trains of stimuli lasted from 50 msec to 10 seconds. The locations of the stimulating electrodes were verified histologically at the end of each experiment by making an electrolytic lesion at the electrode tip which could later be identified on serial sections of the brain stem stained with cresyl violet.

Tungsten microelectrodes with impedances of 1 to 3 megohms were used to record single-unit dorsal-horn cell activity. The criteria for identification of a unit as a cell and not an afferent fiber have been described previously.25,26 The receptive field of and adequate stimulus for exciting each dorsal-horn cell were recorded. Reproducible cutaneous stimuli were delivered with either an electromagnetically driven stylet (producing hair movement) as the innocuous stimulus or with a contact Peltier thermode applied to the shaved hairy skin or foot pad (delivering temperature pulses up to 54°C) as the noxious stimulus. When the noxious paradigm was used, the stimuli were applied no more frequently than every 5 minutes in order to decrease the effects of skin sensitization. The times of occurrence of the extracellularly recorded dorsal-horn unit action potentials as well as the timing and magnitude of cutaneous and brain-stem stimuli were collected with a
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PDP-11/34 or a Hewlett-Packard 1000/A900 computer.

Following determination of a unit's control response to either the innocuous or noxious stimuli, the effects on the response of stimulating the KF were evaluated. While the significance of the effects of KF stimulation on the responses of a unit to innocuous skin stimulation could easily be determined using Student's t-test, the skin sensitization following application of repeated noxious heat stimuli to an area of skin made the use of such statistics in the noxious stimulation paradigm unreliable. Therefore, the threshold for inhibition of a unit's response to noxious stimulation was considered to be the lowest level of brain-stem stimulation that caused an obvious concomitant decrease in the unit's ongoing firing rate. This almost certainly results in an overly high estimate of the brain-stem stimulation threshold amplitude. Latency of stimulation effects was determined by activating the unit under study with a continuous noxious heat stimulus (usually 50°C to 52°C). Then 50 to 100 repetitive trains of brain-stem stimuli (5 pulses at 100 Hz) were delivered every 7 seconds. Peristimulus histograms were constructed and, from these, the method of cumulative sums was used to identify the first trend after brain-stem stimulation that indicated a decrease in activity. This technique is particularly useful in identifying trends of activity when the response is variable, as is seen in typical neuronal spike train recordings. The least-squares technique was used to plot the best straight line fitting this trend. The point where this line crossed the zero activity line was then considered the onset of inhibition. The time difference from the beginning of the brain-stem stimulus to the onset of inhibition is the latency of the brain-stem stimulation effect.

The effects of depleting central nervous system (CNS) monoaminergic stores were determined in five cats. These animals were pretreated with reserpine, 1 to 2 mg/kg intraperitoneally 24 hours before the acute experiment. The effectiveness of the dose of reserpine in depleting CNS catecholamines was evaluated by processing the post-experimentation tissue for catecholamine histofluorescence using the glyoxylic acid technique. In animals so tested, the reserpine was effective in totally abolishing spinal cord histofluorescence.

The results were analyzed statistically by computer.

**Results**

The effects of stimulating the KF were determined for 11 dorsal-horn cells responding only to innocuous cutaneous stimulation and for 21 dorsal-horn cells responding exclusively or maximally to noxious cutaneous stimulation. Stimulation of the KF potently inhibited the responses of the dorsal-horn cells responding only to innocuous stimulation and similarly inhibited the responses of all but one of the cells tested with noxious skin stimulation. The single exception was a lamina-7 cell that responded to pinch and noxious temperature as well as proprioceptive input, and which was facilitated by KF stimulation. The mean threshold for inhibiting the responses to noxious skin stimulation (28.75 μA) was lower than that for inhibiting responses to innocuous skin stimulation (58.18 μA). Analysis of variance indicates that the difference between the thresholds for inhibiting responses to noxious and innocuous skin stimulation was significant (F = 4.599, p = 0.038).

**FIG. 2.** The effects of Köllinger-Fuse nucleus (KF) stimulation on dorsal-horn unit responses to innocuous skin stimulation. A: The amplitude and time course of the cutaneous displacement stimuli used to elicit the responses shown in B and C. B and C: Peristimulus time histograms constructed from 20 consecutive trials each. The bin width is 25 msec. The control response elicited from the dorsal horn neuron by the illustrated cutaneous stimulus is shown in B, and the inhibitory effects of 50-μA stimulus trains delivered at KF is illustrated in C. The black bar indicates the time course of the brain-stem stimulus trains. D: The receptive field of the neuron.
The effects of low stimulus strength activation of the Kölliker-Fuse nucleus (KF) on the responses of a dorsal horn unit to cutaneous innocuous stimulation. A: The time course and amplitude of the cutaneous displacement used to elicit the responses illustrated. B-D: Peristimulus time histograms constructed from 20 consecutive trials each: control response (B), inhibition resulting from a 50-μA KF stimulus (C), and inhibition resulting from a 5-μA KF stimulus (D). The black bars indicate the time course of the stimulus trains. The double asterisk indicates a significant change from control response (t-test, p < 0.001).

The effects of Kölker-Fuse nucleus (KF) stimulation on the responses of a dorsal horn unit to noxious thermal skin stimulation. A: The magnitude and time course of the thermal stimulus applied to the skin to elicit the responses shown below. B: The control response. C: The inhibitory effect of KF stimulation on the unit's response to the noxious thermal stimulus. The times of the brain-stem stimuli are indicated by the lines of small triangles. The bin width for all the histograms is 500 msec.

Elicitation by KF stimulation is demonstrated in Fig. 5. Clearly, relatively minor repositioning of the brain stem-stimulating electrode by as little as 0.5 mm can result in loss of the inhibitory effect of the KF stimulus when delivered at levels near threshold. The locations of the KF stimulation sites determined by examination of the electrolytic lesions made at the site of stimulation at the end of each experiment are shown in Fig. 6. Results from experiments in which the stimulation site was not within the KF area were discarded. The effective thresholds for inhibition of dorsal-horn unit responses by stimulation in areas outside the KF were higher than the thresholds found within the KF. Figure 6 also illustrates some of the recording sites, determined by electrode track reconstruction, of units inhibited by KF stimulation. It can be seen that the locations of units inhibited by KF activity are located in all the major sensory laminae of the dorsal horn. However, there were no lamina-1 units included in this study.

The typical time course of the effects of KF stimulation on the activity of a dorsal horn unit responding to noxious thermal stimulation is given in Fig. 7 upper left. The maximum inhibition occurred 275 msec after the onset of the 50-msec brain-stem stimulus, and the duration of the inhibition was in the range of 1 second. The cumulative sum histogram (Fig. 7 upper right) shows the presence of inhibition as a negative curve, and the resumption of the control rate of neuronal firing as a flattening of the curve to a nearly zero slope. Not all units displayed such a prolonged inhibitory period. The inhibition of another unit is illustrated in Fig. 7 lower; the unit showed a much shorter time course with the maximum inhibition occurring 175 msec after the 50 msec KF stimulation and lasting only about 500 msec. This unit displayed a fairly prominent postinhibitory period of increased activity, which is reflected as a positive slope of the cumulative sum histogram (Fig. 7 lower right). When the cumulative sum histogram is plotted with a short bin width (2 msec), an accurate estimate of the latency, and therefore of the conduction velocity of the descending inhibitory system, can be obtained (Fig. 8). The latencies for KF inhibition of dorsal-horn units ranged from about 60 to 75 msec, indicating bulbospinal conduction velocities of 4 to 5 m/sec.

Reserpine treatment was very effective in accomplishing spinal cord CA depletion; no spinal CA's could be identified in the treated animals using a sensitive histofluorescence technique. Recordings were made from five cells responding only to innocuous hair movement and from 40 cells responding either exclusively or
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FIG. 5. The effects of small changes in the stimulating electrode site on the Kölliker-Fuse nucleus (KF) stimulation-induced inhibition of a dorsal-horn cell response to noxious thermal skin stimulation. A: The time course and magnitude of the cutaneous thermal stimulus used to elicit the responses illustrated in the histograms. B: The control response. C–H: The effects of 50-μA trains of stimuli (triangles) applied to the brain stem in the locations relative to the KF as indicated. The inhibitory effect is most clear when the stimulus is delivered at or within 0.5 mm of the KF.

Differentially to noxious thermal cutaneous stimulation in these monoamine-depleted animals. There was a dramatic change in the ability of KF stimulation to inhibit the responses of both types of units to cutaneous stimulation. Only one of the five cells responding to innocuous stimuli could be inhibited with stimulus strengths of less than 100 μA, and only two of the 40 cells responding to noxious skin stimulation could be inhibited by KF stimulation strengths of less than 50 μA. Of the remaining cells, 15 were either not inhibited or were facilitated by KF stimulation at 50 μA or greater. For those units with responses to noxious skin stimulation that could be inhibited the mean threshold was 129.7 μA, compared to 28.75 μA for the cells from animals with intact CA stores. The inhibitory effectiveness of KF stimulation in the two groups (control and reserpine-treated) was significantly different as shown in Table 1 (chi-square test with Yates' modification, p < 0.0001).

Discussion

These data indicate that stimulation in the region of the KF results in potent inhibition of the responses of dorsal-horn cells to both noxious and innocuous stimuli. The thresholds for this inhibitory effect are low, commonly being in the range of 5 to 10 μA, a level that implies there is very little spread of the effective stimulus.36 This assumption is confirmed by the loss of inhibitory effect when the stimulation site is varied by as little as 0.5 mm. While it is tempting to conclude from the threshold data that this system preferentially inhibits dorsal-horn cell responses to noxious stimula-
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FIG. 6. Composite drawings. Left: The Kölliker-Fuse nucleus (KF) stimulation sites for some of the experiments in this study are shown (solid circles). SC = nucleus subceruleus; LC = locus ceruleus. Right: Recording sites. The solid circles represent the locations of units responding only to innocuous stimuli and inhibited by KF stimulation. The open circles represent the locations of units responding preferentially to noxious stimuli and inhibited by KF stimulation. The triangles represent the locations of nociceptive specific cells that were inhibited by KF stimulation.

...tion, this conclusion is not necessarily valid, since it has been shown that the stimulus response paradigm used can affect, to some extent at least, the determination of threshold. This occurs because it is easier to demonstrate inhibition in a system with a brisk response (as in the noxious thermal paradigm). Even so, like other systems associated in nociceptive control mechanisms, the KF-spinal system inhibits responses to noxious cutaneous stimuli at thresholds lower than that required for inhibition of responses to innocuous cutaneous stimulation.

Biogenic amine depletion with reserpine significantly altered the effects of KF stimulation both by increasing the threshold for inhibition of some cells and by increasing dramatically the proportion of cells either not inhibited or actually facilitated as a result of this stimulation. This suggests that the inhibitory effects of the KF-spinal system are partially dependent on intact central stores of noradrenaline, a finding consistent with the prior demonstration of the KF noradrenergic innervation of the spinal cord. Noradrenergic dependence is further suggested by the relatively low conduction velocity measurements. Catecholaminergic systems have conduction velocities in the unmyelinated fiber range (less than 1 m/sec). The conduction velocities that are reported here are somewhat faster than the unmyelinated fiber range, but are slower than that reported for reticulospinal fibers (between 20 and 100 m/sec). This implies that the inhibitory effects that we have seen are mediated either by small myelinated fibers or by a mixture of unmyelinated and myelinated fibers. This possibility is compatible with the anatomical studies showing a high concentration of CA terminals in the dorsal-horn apex, the primary source of which is the KF. However, following reserpine treatment, there is not a complete loss of inhibition. This indicates that a heterogeneous descending system may be involved in eliciting the inhibitory effects that were observed, a finding consistent with the anatomy of the dorsolateral pons.

Detailed studies of the projection systems from the dorsolateral pons to the spinal cord indicate that KF is the primary, but not the only, source of descending CA fibers that terminate in the spinal cord. Furthermore, the areas within the pons that supply CA's to the spinal cord also send many non-CA terminals to the same area. The contribution provided by non-CA systems from the dorsolateral pons to antinociceptive effects can be extremely potent, however, their relative contribution to the KF-spinal effects is not certain. This is of some importance since CA's have been shown to be necessary for the full expression of opiate analgesia as well as some varieties of stimulation and behavior-induced analgesia. Locus ceruleus stimulation results in behavioral analgesia and suppression of segmental neuronal responses to a variety of peripheral stimuli. The assumption that LC is responsible for spinal noradre-
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**FIG. 7.** The time course of Kölliker-Fuse nucleus (KF) stimulation effects on the response of two dorsal horn units to noxious thermal skin stimulation. The bin width for these plots is 50 msec. **Upper Left:** Peristimulus time histogram made from 100 consecutive trials during which the skin temperature was maintained at 52°C. Each bin contains the total number of spikes recorded in 100 trials (average/bin: 72.22). The KF was stimulated with 75 μA at a rate of 100 Hz for 50 msec (black bar). The activity used as a control response was obtained just before the onset of the KF stimulation (criss-crossed box). Cross-hatched box = the time of maximum inhibition (at 275 msec); **horizontal line** = average control response. **Upper Right:** Cumulative sum plot of the same data. Each bin is a cumulative sum of the variations from the control response, calculated by subtracting the control response from the stimulation bin value and then adding this value to the sum of the previous deviations from the control response. Consequently, a trend of even small decreases in consecutive stimulation bin values will show up as a cumulative sum plot with a negative slope, while a lack of consistent changes from the control values will appear as a cumulative sum plot with zero slope. The magnitude of the deviations from the control response level is indicated by the slope of the cumulative sum plot. Here the control average is 0, and a negative curve represents less than average activity, while a positive curve would indicate a greater than average activity. **Lower:** Peristimulus time histogram (left) and cumulative sum (right) constructed exactly as in the upper graphs from 60 consecutive trials in a second unit (average spikes/bin 36.22). This unit showed a much shorter time to maximum inhibition (175 msec), followed by a period of increased activity.

**TABLE 1**

**Effects of reserpine on KF stimulation-induced alterations of dorsal-horn cell responses to noxious skin stimulation**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. Units Inhibited by KF Stimulation</th>
<th>Other Units†</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>reserpine-treated</td>
<td>2</td>
<td>38</td>
</tr>
<tr>
<td>expected results</td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>6.67</td>
<td>13.33</td>
</tr>
<tr>
<td>reserpine-treated</td>
<td>13.33</td>
<td>26.67</td>
</tr>
</tbody>
</table>

*KF = Kölliker-Fuse nucleus. Degrees of freedom = 1; χ² = 43.0094; p < 0.00001.*

† Other units: units inhibited at > 50 μA KF stimulation or with uninhibited or facilitated responses.

**FIG. 8.** Latency determination for Kölliker-Fuse nucleus (KF)-induced effects on a dorsal-horn unit response to noxious thermal skin stimulation. The method used for calculating the cumulative sum plot and latency is described in the text. The time of KF stimulation is indicated by the black bar, and the time of onset of inhibition is shown by the short vertical line. Number/bin refers to spikes/bin; bin width was 2 msec. In this case the latency was 70.05 msec.

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neric effects is untenable, for the cat at least, in view of anatomical evidence.\textsuperscript{43} There are differences between the effects of LC stimulation and those of KF stimulation on the responses of dorsal-horn neurons. The effects of KF stimulation have a longer latency than effects of LC stimulation and are significantly decreased by prior reserpine treatment, while LC inhibition is not decreased by reserpine pretreatment.\textsuperscript{22} Further, few CA-containing LC cells project to the spinal cord,\textsuperscript{44} and dorsolateral pontine lesions in the region of the LC interrupt morphine analgesia without decreasing spinal CA levels.\textsuperscript{20}

It seems likely than that the KF-spinal pathway can account for much of the bulbospinal catecholaminergic effects that play an important theoretical and practical role in analgesic systems. It is clear that the relatively simple descending system originally described by Basbaum, et al.,\textsuperscript{3,4,44} as the anatomical and physiological substrates for opiate and other pain control systems must be expanded to include nuclei and projections other than those involved in the serotonergic raphe-spinal system and the LC-spinal system. It is not clear whether there is clear functional specificity in the role that these different descending systems assume in the functioning animal. Catecholaminergic brain-stem systems may also contribute to the overall importance of monoamines in antinociception\textsuperscript{1} because of their interconnections with other nuclei projecting directly to the spinal cord.\textsuperscript{38}

The existence of the basic pharmacological machinery present in the dorsal horn that allows dorsal-horn cell responses to noxious skin stimulation to be inhibited by a descending CA pathway suggests that direct manipulation of this system using intrathecal drugs might provide a means of treating a variety of pain states with nondestructive and non-narcotic alterations.\textsuperscript{24,33}

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References

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