

Suppression of Noxiously Evoked WDR Dorsal Horn Neuronal Activity by Spinally Administered Morphine

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The present study was carried out in order to examine the ability of spinally administered morphine to suppress noxiously evoked activity of wide-dynamic-range (WDR) neurons in the dorsal horn of the spinal cord in decerebrate, spinal cord-transected cats. All cells ($n = 25$) responded maximally to high-intensity noxious heat stimulation (51°C) and were classified as wide dynamic range neurons. The spinal administration of 0.1 mg of morphine caused a significant reduction of noxiously evoked activity but did not significantly change spontaneous activity. The 0.25-mg dose caused a significant reduction of both types of activity. Thirty minutes after spinal administration, 0.1 mg of morphine caused a 27% reduction of spontaneous activity and a 43% reduction of noxiously evoked activity. The 0.25-mg dose reduced spontaneous activity by 44% and the evoked activity by 70%. Naloxone partially reversed the morphine-induced neuronal suppression. In addition, in the four neurons in which it was tried, spinally administered epinephrine was found to further suppress the remaining neuronal activity following the spinal morphine effect. These results demonstrate for the first time that spinally administered morphine is capable of suppressing noxiously evoked activity of wide-dynamic-range neurons in the dorsal horn of the spinal cord. They also demonstrate the dose-dependent nature of this effect and the potential importance of the interaction between morphine and adrenergic agonists in blocking information about noxious events. This information provides a probable mechanism of action for spinal opiate analgesia. (Key words: Analgesia. Anesthetic techniques: spinal morphine. Pain: noxious heat. Spinal cord: WDR neurons.)

THERE HAS BEEN GREAT INTEREST within the last several years in the subarachnoid or epidural application of opiates for the production of analgesia for pain of both acute and chronic origin. The development of spinal opiate analgesia provides an excellent example of the important relationship that exists between basic science and clinical research. A fairly short period of time passed between initial reports that systemic or iontophoretic opiate administration modulated afferent spinal cord activity¹⁻⁴ and the first reports in animals⁵ and humans⁶ that the spinal administration of morphine could produce analgesia. Because of the great potential importance of spinal opiate analgesia, it is essential that

carefully controlled studies be performed, both in the clinic and in the basic science laboratory, in order to adequately define the potentials and the limitations of this new and promising technique. This study was undertaken to examine the dose-response relationships, onset, duration, and potential naloxone reversibility of suppression of dorsal horn wide-dynamic-range (WDR) neurons by spinally administered morphine. The spinal administration technique was designed in order to closely simulate clinical spinal analgesia.

Methods

Experiments were performed on 25 cats of either sex. Surgical preparation was carried out under halothane-nitrous oxide, oxygen anesthesia. Following the placement of an external jugular vein catheter for fluid administration, bilateral carotid artery catheters for blood pressure monitoring and blood sampling, and an endotracheal tube for controlled respiration, the animals were rendered decerebrate with electrolytic lesions in the midbrain reticular formation and paralyzed with gallamine. Anesthesia was then discontinued, a laminectomy was performed at L4 through L6, and the spinal cord was transected at T12. The dura was incised, and a tungsten microelectrode was advanced into the spinal cord in order to obtain extracellular, single unit recordings of wide-dynamic-range neurons in the dorsal horn of the spinal cord. Physiologic parameters were monitored and maintained within normal limits. During the search procedure, the spinal cord was bathed with 37°C physiologic saline.

When a single neuron was located, the cell type was identified by the depth of the electrode, by the spontaneous firing pattern, and by its response profile to peripheral stimuli of the following types: 1) air puff; 2) light touch with a camel's hair brush; 3) noxious pinch with a forceps; 4) noxious radiant heat; and 5) application of ethyl chloride. All neurons which responded with a WDR profile [*i.e.*, responded with increasing activity to stimuli of increasing intensity (from the non-noxious to the noxious range) with the greatest response occurring during the application of a noxious stimulus] were included in this study. Heat stimuli were produced by a radiant heat source which was directed at the receptive field of the cell under study. A thermocouple was placed adjacent to the center of the receptive field

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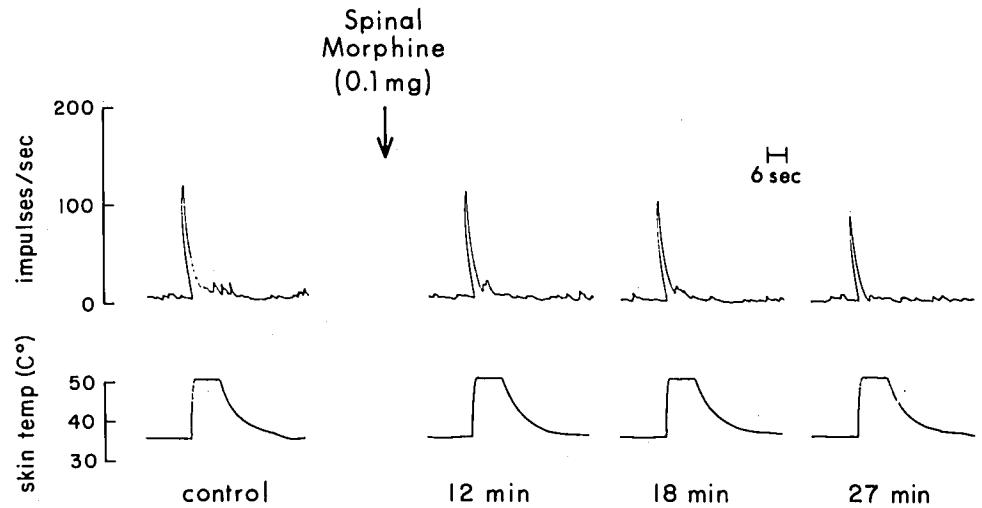
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FIG. 1. Effects of a single dose of 0.1 mg of spinally administered morphine on activity of an individual WDR neuron. The top tracings indicate a rate meter output of neuronal activity expressed as impulses per second. The bottom tracings indicate the skin temperature beneath the radiant heat stimulus as recorded by a copper-constantan thermocouple which also served as the feedback control for the heat stimulus. The stimulus was turned on at the initial rise in the deflection from baseline and remained on for a period of eight seconds. The turning off of the stimulus allowed the skin to cool passively. In each instance, a 51° C radiant heat stimulus was presented. The left figures indicate that in the control situation, the presentation of a 51° C radiant heat stimulus for eight seconds caused an obvious activation of this WDR neuron, a neuron which had very little spontaneous activity. Following the spinal administration of 0.1 mg of morphine, there was, with time, some decrease in the evoked activity of this particular neuron.



to monitor the skin temperature changes and provide feedback control to maintain a constant temperature.

Following the isolation of a single cell and control studies, the physiologic saline which had been bathing the spinal cord was removed, and either 0.1 or 0.25 mg of morphine, dissolved in either 0.3 or 0.5 ml of saline, was dropped gently onto the spinal cord. [This solution contained not more than 0.03% of preservatives (chlorbutanol, sodium bisulfite) which were present in the morphine used for solution preparation.] This volume was adequate to cover the spinal cord without providing a large enough volume to rise above the bony protuberance of the spinal column and come in contact with the highly vascularized muscle. Control studies ($n = 5$) were performed using preservative-free morphine (0.25 mg). The cord was monitored microscopically to insure that it did not dry out, and additional small volumes of drug free saline were placed on the spinal cord if necessary. Following the administration of morphine on the spinal cord, electrophysiologic recordings were carried out for a period of up to 150 min.

The ability of naloxone to reverse morphine effects also was examined. In most experiments, naloxone (0.1–0.4 mg) was placed on the spinal cord 30 min after spinal morphine administration. In several experiments, 60 min passed between the spinal application of morphine and naloxone. In some instances, an additional intravenous dose of naloxone (0.1–0.4 mg) was administered after the spinal dose.

Both spontaneous and evoked activity were recorded during control studies and following spinal drug administration. Evoked activity was elicited by the application of an eight-second, 51° C radiant heat stimulus to the neuron's receptive field on the foot pad. Data

were collected on-line with a digital computer (DEC PDP 11/40) for subsequent off-line analysis. The spontaneous discharge frequency of each cell was determined by averaging the discharge frequencies observed for 20 s before each exposure to heat. The mean evoked discharge frequency of each unit was determined by averaging the discharge frequency observed for 10–20 s after each exposure of the receptive field to radiant heat. The statistical significance of differences between mean values was assessed by Student's *t* test for paired data (for comparison of changes from the control in each experiment) and Student's *t* test for unpaired data (for comparison of effects between 0.1 mg and 0.25 mg of morphine).

Results

All the neurons included in this study ($n = 25$) were of the WDR type.

Figure 1 shows the effects of 0.1 mg of spinally administered morphine on the neuronal activity of an individual WDR neuron. The 0.1-mg dose caused a reduction in neuronal activity of this cell but the reduction was not as large as that seen in cells following 0.25 mg of spinally administered morphine.

Figure 2 demonstrates the effects of 0.25 mg of spinally administered morphine on both the spontaneous and stimulus-driven activity of a single, WDR neuron recorded from the dorsal horn of the spinal cord. Following the spinal administration of morphine, there was a significant reduction in both types of activity, the reduction being more apparent in the evoked activity.

Figure 3 shows the depressant effect of 0.1 mg and 0.25 mg of spinally administered morphine on the mean

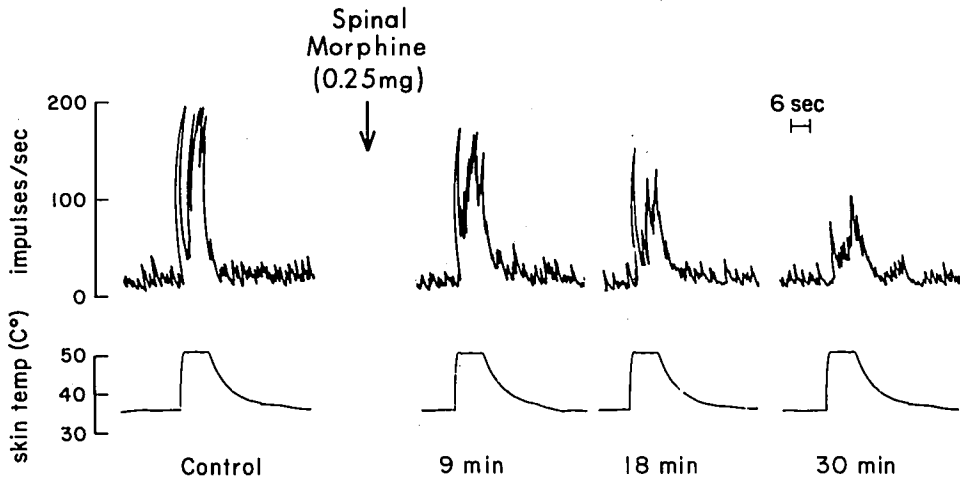


FIG. 2. Effects of a single dose of 0.25 mg of spinally administered morphine on activity of an individual WDR neuron (See figure 1 legend for details). The control tracings on the left indicate that the presentation of a 51° C radiant heat stimulus for eight seconds caused activation of this WDR neuron, a neuron which had spontaneous activity. Following the application of 0.25 mg of morphine on the spinal cord, there was, over time, an obvious reduction in the evoked activity with some reduction in the spontaneous activity.

evoked WDR neuronal activity of all the cells that were suppressed in this study. At the 30-min time point the evoked activity had been reduced to approximately 63% (0.1 mg) and 30% (0.25 mg) of control values, respectively. Spontaneous activity was suppressed to a lesser extent by both doses than was the stimulus-driven activity. Thirty minutes after morphine administration, the spontaneous activity was reduced to 73% (0.1 mg) and 56% (0.25 mg) of control values, respectively.

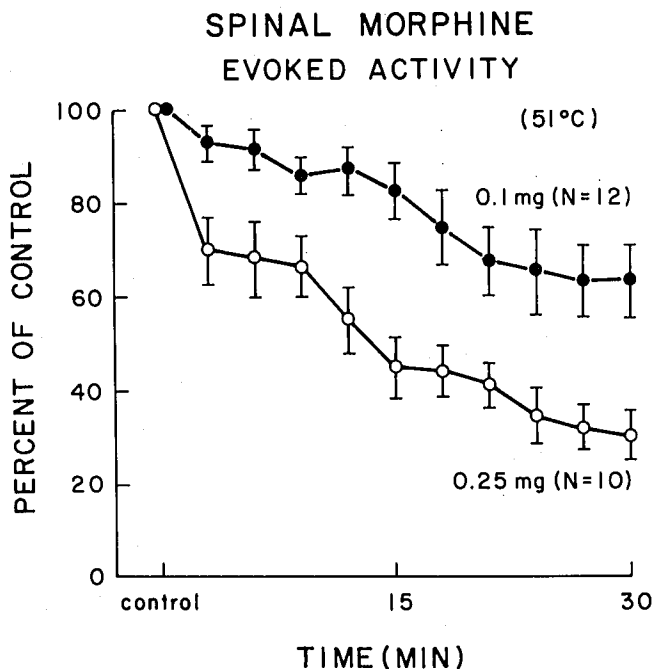


FIG. 3. Effects of 0.1 and 0.25 mg of spinally administered morphine on the mean evoked activity of all neurons that were suppressed in this study. Abscissa is time in minutes; ordinate is neuronal activity expressed as a per cent of control. This figure demonstrates that there was a dose-response effect of spinally administered morphine on evoked neuronal activity.

The statistical significance of the observed changes are seen in table 1. The 0.1-mg dose caused a significant reduction in the evoked activity at both 15 and 30 min. The 0.25-mg dose caused a significant reduction in both types of activity. There was a statistically significant dose-response effect on the evoked activity ($P < 0.01$) but not on the spontaneous activity. Two neurons were initially slightly excited by 0.1 mg but were depressed by that same dose at 30 min. In addition to the 22 cells that were suppressed, three cells were unchanged by the 0.25-mg dose. These neurons were not included in the population reported in this study.

The spinal administration of naloxone produced a partial reversal of the morphine-induced suppression. Figure 4 demonstrates an example of that reversal. In no instance was complete reversal produced by the spinal administration of naloxone. Subsequent intravenous administration of naloxone enhanced the rever-

TABLE 1. Mean Suppressive Effects of Spinal Morphine on Dorsal Horn WDR Neuronal Activity

Dose of Morphine	Control	15 Minutes	30 Minutes
0.1 mg (n = 12)			
Spontaneous activity	11.5 ± 2.0	9.47 ± 2.1 (82%)	8.4 ± 1.9 (73%)
Evoked activity	54.7 ± 9.5	46.3 ± 9.6* (85%)	31.2 ± 7.2* (57%)
0.25 mg (n = 10)			
Spontaneous activity	23.0 ± 4.8	15.4 ± 3.7* (67%)	13.0 ± 2.9* (57%)
Evoked activity	72.9 ± 8.7	34.7 ± 6.1† (48%)	22.2 ± 4.3† (30%)

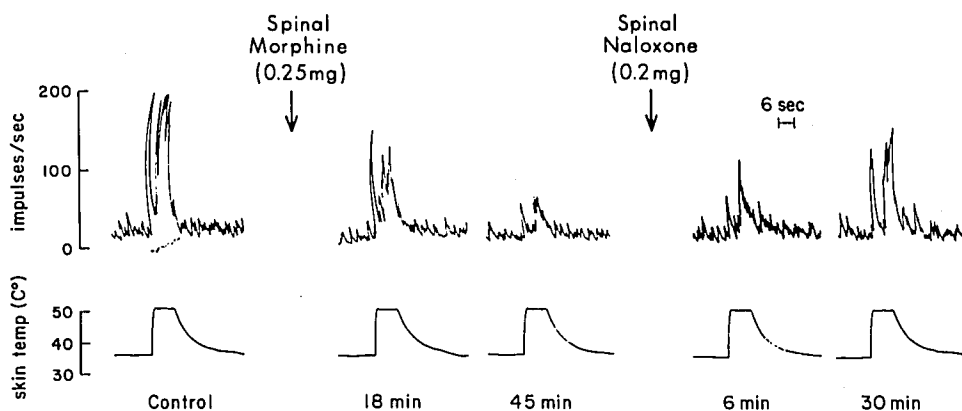
Activity is expressed as impulses per second.

Numbers in parentheses indicate per cent of mean control neuronal activity.

* $P < 0.01$.

† $P < 0.001$.

FIG. 4. An example of neuronal suppression by spinally administered morphine of a WDR neuron and partial reversal of that suppression by naloxone. The left panel indicates that during the control situation, a 51° C radiant heat stimulus for eight seconds caused obvious activation of this WDR neuron. The spinal administration of 0.25 mg of morphine caused a suppression which was present even at 45 min after drug administration. The subsequent spinal administration of 0.2 mg of naloxone caused a significant reversal of the morphine suppression of this particular WDR neuron.



sal in some instances but again did not bring about complete reversal.

In the neurons in which activity was recorded for up to one hour prior to the administration of naloxone, there was no indication of recovery of activity following the administration of the 0.25-mg dose of morphine (*i.e.*, each neuron was firing at a suppressed rate 60 min after spinal morphine administration).

The effects of 0.25 mg of preservative-free morphine were similar to those produced by the preservative-containing solution. No significant difference was noted in the onset, duration, or degree of suppression or in the naloxone reversibility of the suppression.

In four cases, epinephrine (10–100 μ g) was applied to the spinal cord following the morphine and naloxone study. The epinephrine caused a significant enhancement of the morphine-induced suppression. There was no obvious change in the gross appearance of the spinal cord under microscopic examination following the epinephrine administration.

Discussion

Spinal opiate analgesia has great potential for the alleviation of many types of pain, and thus, it is imperative that we obtain a detailed understanding of its mechanism of action and potential side effects. The present study was conducted in order to determine if the spinal administration of morphine could produce a dose-dependent suppression of noxiously evoked activity of spinal neurons which are thought to be associated with pain. We also were interested in determining the relative time course of the suppression of neuronal activity as compared with the systemic administration of morphine. In addition, we wanted to determine the relative susceptibility of this suppression to reversal by both spinally and systemically administered naloxone.

Wide-dynamic-range neurons were studied because of the evidence suggesting that they are associated with the transmission of information about pain.^{7,8} These neurons not only respond maximally to noxious stimuli but also have been identified as being cells of origin for the spinothalamic tract.^{7,9} The use of radiant heat enabled a precisely controlled, quantifiable, noxious stimulus (*i.e.*, with no mechanically superimposed non-noxious component) to be presented to the receptive field and to activate the neurons under study.

One of the important points of this report is the fact that noxiously evoked activity was suppressed by morphine. It had previously been reported that spinally administered morphine was capable of suppressing spontaneous activity of WDR neurons.¹⁰ However, because the underlying causes of spontaneous activity are not understood clearly, it was imperative that the suppression of activity caused by a clearly painful stimulus be demonstrated. Suppression of noxiously evoked activity as indicated in the present study demonstrates a clear mechanism of action for the production of spinal opiate analgesia.

The significance of the present study is the demonstration that there is a dose-response relationship between the amount of morphine placed on the spinal cord and the amount of suppression of noxiously evoked activity of WDR neurons. This existence of a dose-response relationship suggests that in clinical work there should be an optimum dose for the alleviation of various types of pain. As with other drugs which interact with specific receptors to produce an effect, it should be possible to determine appropriate doses for spinal opiate analgesia.

The time course of the neuronal suppression by spinally administered morphine, as observed in this study, supports the clinical observations that spinal opiate analgesia is of long duration. It would appear that the long

duration is due to a prolonged suppression of spinal dorsal horn neurons. An earlier study from this laboratory³ indicated that a 1.0 mg/kg intravenous dose of morphine (a dose approximately 16 times greater than the maximum dose used in the present study) suppressed WDR neurons, and the suppression lasted for a period of approximately 30 min. In the present study, however, the spinal administration of morphine resulted in suppression of neuronal activity with no spontaneous recovery for periods of up to 60 min after the administration of morphine. Recovery was seen only after naloxone administration. A comparison of these results would suggest that the duration of spinal cord neuronal suppression by spinally administered morphine is much greater than the duration following systemically administered morphine.

The degree of naloxone reversal in the present study raises several important questions. The first interpretation would be that the placing of morphine on the spinal cord produces very high concentrations of the drug near the receptors, thus making it more difficult for naloxone to cause reversal. This interpretation would be in keeping with anecdotal reports in the clinical literature that in those patients in whom spinal opiate administration caused respiratory depression, subsequent administration of naloxone was capable of reversing the respiratory depression without causing any apparent change in the patient's pain sensitivity.^{11,12}

A second consideration relative to the naloxone reversal concerns the important concept of how much suppression of neuronal activity must occur before there is a detectable change in the sensation of the individual. Unfortunately, we do not have the answer to this question. We are capable of indicating whether or not a change is statistically significant, but we do not know if a particular amount of suppression of an individual neuron or a group of neurons will be detected by the animal as a change in sensation. Therefore, although we were not able to demonstrate complete reversal of the morphine effects at the level of the spinal cord, whereas others have shown behavioral reversal of analgesia following spinal naloxone administration,¹³ it is quite possible that the amount of reversal of neuronal effects as seen in the present study, were adequate for the reversal of behavioral analgesia.

These studies were carried out with a morphine solution that contained an extremely low concentration of preservatives. The lack of preservative effect is substantiated by both the control studies with preservative-free morphine and the fact that naloxone produced significant reversal of the spinal morphine effect. Naloxone reversal indicates that the neuronal suppression was due in large part to interaction of morphine with specific opiate receptors.

In summary, these results demonstrate that the spinal administration of morphine is capable of suppressing noxiously evoked activity of WDR neurons. This suppression of noxiously evoked activity is a probable mechanism of action of spinal opiate analgesia.

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