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Effects of systemic morphine on the activity of convergent neurons of spinal trigeminal nucleus oralis in the rat

Radhouane Dallel *, Philippe Luccarini, Jean-Louis Molat, Alain Woda

Laboratoire de Physiologie Oro-Faciale, Faculté de Chirurgie Dentaire, 11 Boulevard Charles de Gaulle, 63000 Clermont-Ferrand, France

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Abstract

The spinal trigeminal nucleus oralis has been shown to relay nociceptive inputs mainly from the oral and perioral regions. In this study, we examined the effects of intravenous administration of morphine on C-fiber-evoked activities of spinal trigeminal nucleus oralis convergent neurons in halothane-anesthetized rats. Morphine depressed the C-fiber-evoked responses of spinal trigeminal nucleus oralis convergent neurons in a dose-related (3–12 mg/kg range) and naloxone-reversible fashion. The ED_{50} was 6.1 mg/kg, a dose similar to that found in the spinal dorsal horn. The observed strong depressive action of morphine on noxious-evoked activities of spinal trigeminal nucleus oralis neurons is consistent with our previous statement, based on electrophysiological studies, that this region plays an important role in the transmission of trigeminal nociceptive information. The effect of morphine on the spinal trigeminal nucleus oralis neurons is discussed in relation to its possible site and mechanism of action.

Keywords: Nociception; Spinal trigeminal nucleus oralis; C-fiber; Morphine

1. Introduction

Recent experiments have suggested that the spinal trigeminal nucleus oralis, the most rostral part of the spinal trigeminal nucleus, is a relay for nociceptive inputs mainly from oral and perioral regions. Arguments for such a role have been provided by clinical, behavioral, anatomical and electrophysiological studies. For example, brain-stem lesions in the vicinity of the spinal trigeminal nucleus oralis may interfere with pain sensations (Graham et al., 1988) or nociceptive behaviors (Young and Perryman, 1984; Broton and Rosenfeld, 1986), elicited by electrical, thermal or mechanical stimuli of oral and perioral tissues. Anatomical studies have shown that the tooth pulp, the stimulation of which elicits predominantely painful sensations, projects heavily to the spinal trigeminal nucleus oralis (e.g., Marfurt and Turner, 1984). Finally, electrophysiological studies have demonstrated the presence of nociceptive neurons which could be functionally classified as convergent (i.e., responsive to both noxious and non-noxious stimuli) and nociceptive-specific (i.e., responsive only to noxious inputs). In this subnucleus, these neurons were activated by mechanical, thermal, electrical or chemical noxious stimuli (Dallel et al., 1990; Raboisson et al., 1991, 1995). The phenomena of diffuse noxious inhibitory controls and of wind-up could also be demonstrated for spinal trigeminal nucleus oralis convergent neurons and their stimulus-response functions, demonstrated with graded mechanical stimulation (Dallel et al., 1990; Raboisson et al., 1995), are consistent with previously documented properties of convergent neurons (see Besson and Chaouch, 1987 for review). These findings suggest that the spinal trigeminal nucleus oralis convergent neurons are capable of providing information regarding the intensity of oro-facial stimuli.

Opiate modulation of nociceptive inputs in the dorsal horn of the spinal cord is well documented (e.g., Duggan and North, 1984; Besson and Chaouch, 1987; Le Bars et al., 1987), but to our knowledge there is limited and conflicting information concerning the spinal trigeminal nucleus oralis. One electrophysiological study has shown that iontophoretically applied morphine does not affect the spontaneous firing of spinal trigeminal nucleus oralis neurons (Andersen et al., 1977). In contrast, morphine microinjected into the spinal trigeminal nucleus oralis depressed the nociceptive behavior induced by a subcutaneous injection of formalin in the upper lip (Luccarini et

* Corresponding author. Tel.: (33) 7343-6414; Fax: (33) 7343-6409.

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al., 1995) or significantly increased the latency of a defensive face-rub reaction in response to noxious facial heat (Rosenfeld et al., 1983). However, no study has, so far, examined the responsiveness of spinal trigeminal nucleus oralis nociceptive neurons to systemic administration of morphine. The purpose of the present study was to investigate the effect of intravenous (i.v.) injection of morphine on C-fiber-evoked activities of spinal trigeminal nucleus oralis convergent neurons, following supramaximal percutaneous electrical stimulation.

2. Materials and methods

2.1. Animal preparation

Experiments were performed on male Sprague-Dawley rats weighing 220–300 g. For surgery, the animals were anesthetized with 2% halothane in a nitrous oxide/oxygen mixture (2:1). After intraperitoneal injection of 100 μg atropine sulfate, a tracheal cannula was inserted, the jugular vein was cannulated, the animals were paralyzed by an i.v. perfusion of pancuronium bromide (0.5 mg/h) and artificially ventilated with a volume-controlled pump (54–55 strokes/min). The levels of halothane, O_2, N_2O and the end-tidal CO_2 (3.5–4.5%) were monitored by an anesthetic gas monitor (Artema MM 200, Sundbyberg, Sweden) during the entire experimental period. These parameters were measured by infra-red absorption, digitally displayed and under the control of alarms. The heart rate was also monitored continuously and core temperature was maintained at 37 ± 0.5°C with a homeothermic blanket system.

The animals were placed in a stereotaxic frame with the head fixed in a ventroflexed position (incisor bar dropped 5 mm under the standard position) by means of an adapted metallic bar. A craniotomy was performed on the right side at the level of the occipitoparietalis suture and the dura mater was removed. After surgery, the level of halothane was reduced to 0.5% and maintained at this level during the recording period. This percentage allowed a level of anesthesia that was adequate for ethical purposes but did not excessively depress neuronal responses to noxious stimuli (Le Bars et al., 1980; Benoist et al., 1984).

2.2. Recordings

Unitary extracellular recordings were made with glass micropipettes (7–10 MΩ) filled with a mixture of 5% NaCl and pontamine sky blue. The brainstem was explored 2.4–3.0 mm lateral to the midline and between the frontal planes, P.1.1 and P.2.6 (Paxinos and Watson, 1986). Single unit activities were amplified and displayed on oscilloscopes and were also led into a window discriminator connected to a CED 1401 interface (Cambridge Electronic Design) and a IBM PS 2 computer, to allow sampling and analysis of the spontaneous and evoked post-stimulus histograms neuronal activity.

Neurons were classified as convergent on the basis of their responses to both mechanical and percutaneous electrical stimulation applied to their receptive field. All neurons responded to both innocuous and noxious mechanical stimuli and showed electrically evoked responses corresponding to both A- and C-fiber inputs (see below).

Innocuous mechanical stimulation of the skin, mucosa and teeth included air puffs, brushing with a soft brush, gentle stroking and light pressure with a blunt probe. Noxious mechanical stimuli consisted of heavy pressure, pinprick and pinching with fine forceps which evoked a painful sensation when applied to the experimenters' skin. Once a neuron had been identified, the extent of its receptive field was determined and mapped and its location was defined in terms of its involvement in intraoral, perioral or more peripheral regions of the face (see Dallel et al., 1990).

Electrical square-wave stimuli (0.66 Hz, 2 ms duration) were applied through a pair of stainless-steel stimulating electrodes inserted subcutaneously into the center of the previously delineated receptive field. The threshold for obtaining a C-fiber response was determined: increasing the current to a suprathreshold value induced reproducible responses. Post-stimulus histograms were analyzed to distinguish responses due to A- and C-fiber inputs, according to their latencies and by using the classification of Gasser and Erlanger (1927) and Burgess and Perl (1973). The latency value of the responses was used to determine the conduction velocity of afferent inputs after making allowance for the conduction distance (40–50 mm) and 1 ms for the central synaptic delay, the delay in activation of the peripheral axons and the narrowing of afferents in the V spinal tract.

At supramaximal electrical stimulation, the responses to either Aβ or Aδ fiber activation could not be distinguished because, due to their short latencies, they overlapped with the stimulus artefact. In contrast, the responses to C-fiber activation were easily recognized, as their long latencies made synaptic delays of little or no consequence. Therefore, since morphine is known to be less potent against the A-fiber responses (Le Bars et al., 1979, Le Bars et al., 1980), only C-fiber-evoked responses were considered in the detailed analysis.

2.3. Experimental design

The experimental procedure consisted of sequences of 50 electrical shocks applied repeatedly (0.66 Hz) to the excitatory receptive field at 3 × threshold for C-fiber activation. This type of stimulation gave an intense and stable response. Sequences were repeated at 5-min intervals.

When two successive control sequences, with a varia-

tion of less than 10% in the magnitude of C-fiber-evoked responses had been recorded, a single dose of morphine hydrochloride (3, 6 or 12 mg/kg) was slowly (2 min) injected intravenously. In each experiment, the mean of the two controls was taken as the reference value for subsequent calculation of the effects of morphine administration on the evoked responses. Inhibition was then expressed as percentage decrease in the number of spikes with C-fiber latencies, with reference to the control. The specificity of the observed effects was tested by injecting the opiate antagonist, naloxone (0.4 mg/kg i.v.).

In each animal, only one spinal trigeminal nucleus oralis cell was tested and one single dose was administered. Only cells showing no alteration in spike amplitude or wave-form during the complete experimental procedure were considered.

2.4. Statistical analysis

The data were analyzed by analysis of variance (ANOVA) followed by the Newman-Keuls test, regression and correlation. The level of significance was set at $P < 0.05$. The results are expressed as means ± S.E.M.

2.5. Histological analysis

Each recording site was marked by electrophoretic deposition of pontamine sky blue. After the animal was killed by injection of a lethal dose of pentobarbital, the brain was removed and fixed in a 10% formalin solution for one week. The tissue was frozen, cut into serial 100 μm thick sections and stained with neutral red. Recording sites (blue dye marks) were determined by microscopic examination and then plotted on camera lucida drawings of serial sections. For illustration purposes, the recording sites were grouped together, according to their position on the rostrocaudal axis, on maps which represented the most frequently observed brain-stem frontal planes.

3. Results

3.1. General properties of convergent neurons

A total of 23 convergent neurons were recorded within the spinal trigeminal nucleus oralis (Fig. 1). They were located throughout the dorsoventral extent of the nucleus, between the frontal planes P 1.1–2.2 (Paxinos and Watson, 1986). The majority of neurons were not spontaneously active. All had an ipsilateral receptive field which included the perioral region (72% of the neurons), the intraoral region (28%) or the peripheral facial region (17%). They were sensitive to both innocuous and noxious mechanical stimuli, and responded by increasing their firing rate as the intensity of the stimuli increased into the noxious range (Fig. 2).

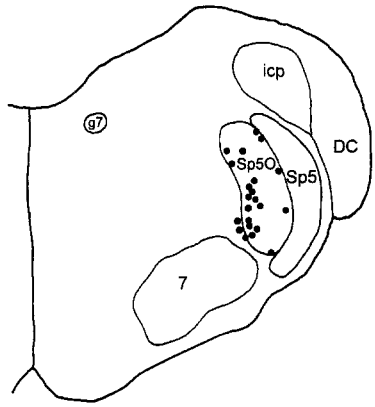

Fig. 1. Histologically confirmed and reconstructed loci of single neurons within the spinal trigeminal nucleus oralis. Locations of spinal trigeminal nucleus oralis neurons are summarized on one representative coronal brainstem plane (P1.7). The nomenclature of the nuclei is based on Paxinos and Watson (1986): DC, dorsal cochlear nucleus, g7, genu facial nerve; icp, inferior cerebellar peduncle; sp5, spinal tract of the V nerve; Sp50, spinal trigeminal nucleus oralis; 7, facial nucleus.

When 2-ms percutaneous electrical stimuli were applied to the center of their excitatory receptive field, responses due to peripheral activation of A- and C-fibers could be observed. Responses due to A-fibers were obtained at a mean threshold of 0.41 ± 0.09 mA and with a mean latency of 3.9 ± 0.6 ms which corresponds to peripheral fibers with conduction velocities in the 10–13 m/s range. The C-fiber responses occurred at a mean latency of 64.4 ± 6.6 ms, corresponding to conduction velocities ranging from 0.6 to 0.8 m/s. The pattern of discharge was unimodal (10/23 cells) or bimodal (13/23 cells). The mean C-fiber threshold was 6.88 ± 0.77 mA. Current intensity was systematically increased to a suprathreshold value that gave reproducible and regular C-fiber responses. At 3 × threshold, a mean of 11.0 ± 1.6 spikes/stimulus was recorded.

3.2. Effects of morphine on C-fiber-evoked responses of convergent neurons

The intravenous injection of morphine produced a depression of the C-fiber-evoked responses of spinal trigemi-

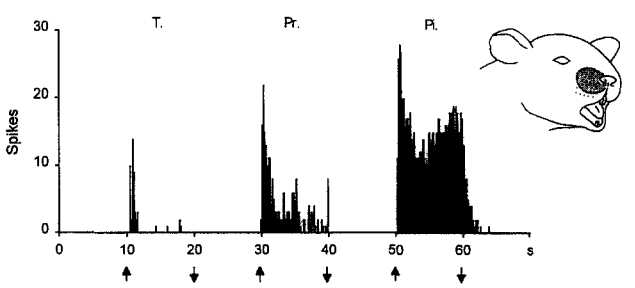

Fig. 2. Individual example of responses of a spinal trigeminal nucleus oralis convergent neuron to mechanical stimuli applied to its perioral receptive filed. The post-stimulus histogram shows the neuron's rapidly adapting response to a constant tactile stimulus (T) applied by a blunt probe to its receptive field, as well as its response to pressure (Pr) and pinch (Pi). Arrows mark the beginning and the end of each stimulation.

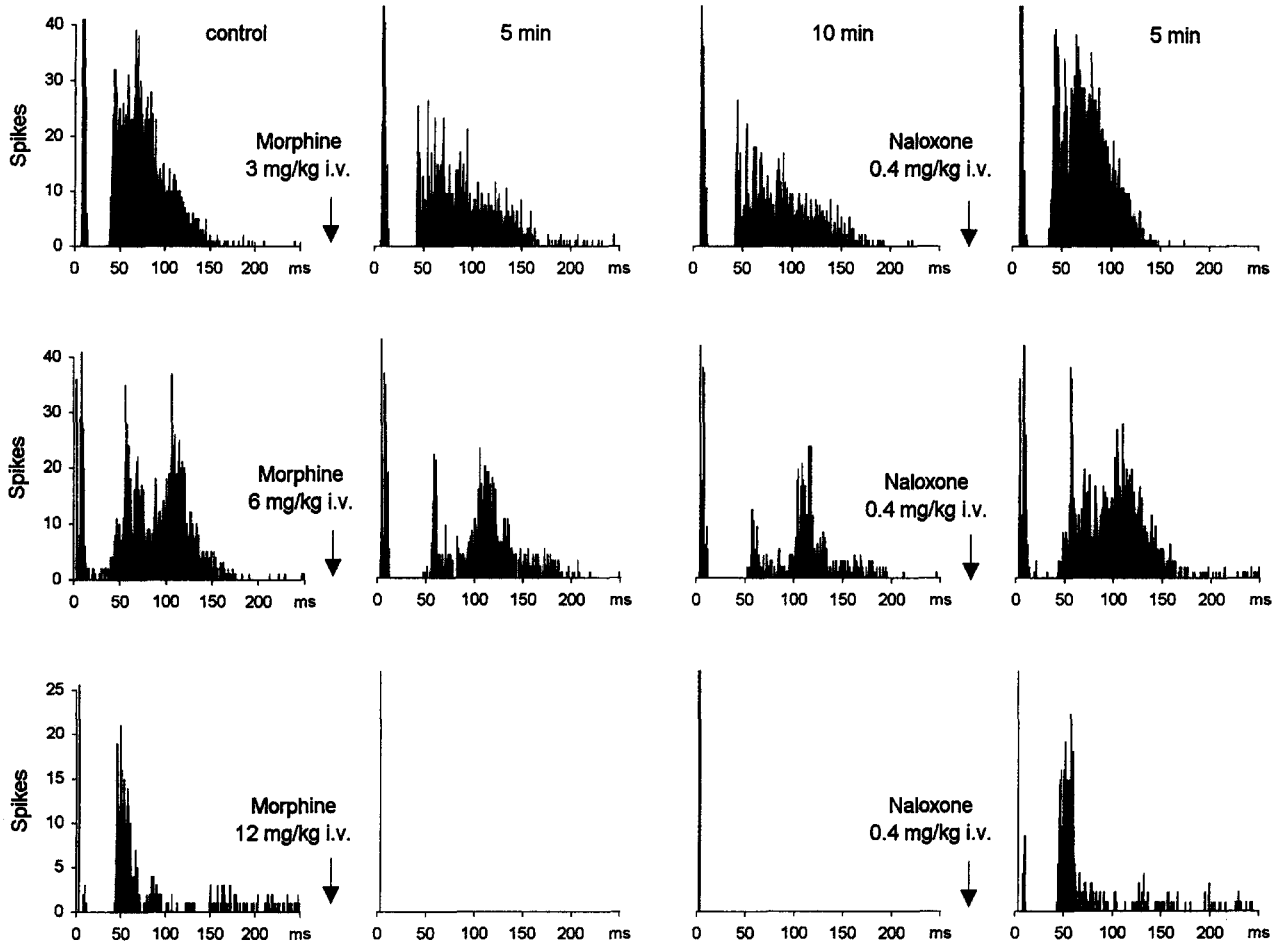

Fig. 3. Individual examples of the effects of three doses of morphine (3, 6 and 12 mg/kg i.v. from top to bottom) on C-fiber-evoked responses of three spinal trigeminal nucleus oralis convergent neurons. Post-stimulus histograms (50 trials) were made before (controls) and after morphine administration as well as following naloxone administration (post injection times are indicated at the top). Note the dose-dependent depressant effect on C-fiber-evoked responses and its reversal by naloxone.

nal nucleus oralis neurons. This is illustrated with individual examples in Fig. 3 which also show that the depression was naloxone-reversible and its size was dose-dependent.

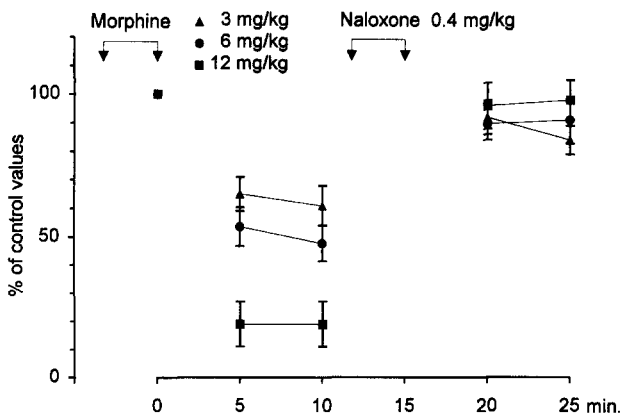

Fig. 4. Cumulative results showing the time course of the effects of three doses of morphine (3, 6 and 12 mg/kg; $n = 8$, 8 and 7, respectively) on C-fiber-evoked responses of spinal trigeminal nucleus oralis convergent neurons. Arrows mark the beginning and the end of the injections. Error bars indicate S.E.M.

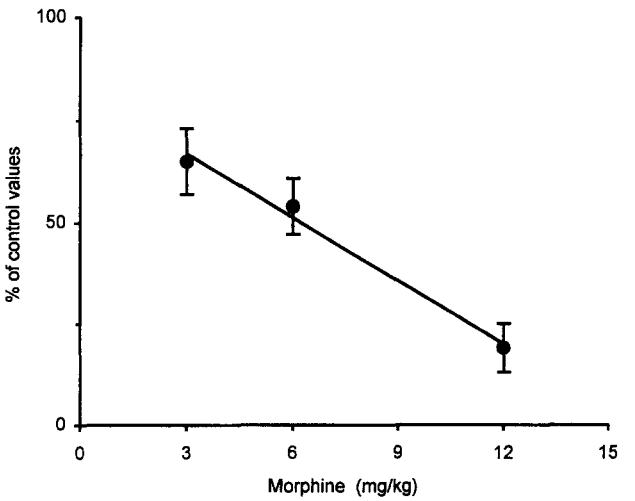

Fig. 5. Dose-related inhibition of the C-fiber-evoked responses for all spinal trigeminal nucleus oralis convergent neurons. The effects of morphine are expressed as percentages of the control responses. The regression line for neurons is $y = -5.04x + 80.7$ ($r = 0.76$).

The cumulative results obtained from 23 neurons are presented in Fig. 4. The depressive effects of morphine on C-fiber-evoked responses were apparent as soon as 5 min after the injection. The responses were reduced to 64 ± 5%, 54 ± 5% and 19 ± 8% of the initial value following 3, 6 and 12 mg/kg of i.v. morphine, respectively. These effects were not significantly enhanced 10 min after injection (Fig. 4) and were always reversed by i.v. administration of naloxone (0.4 mg/kg) (Fig. 3 and Fig. 4). When a bimodal response was observed (Fig. 3), morphine depressed both components.

There was a linear and highly significant relationship between the dose of morphine and the subsequent depression of the neuronal responses (Fig. 5). We calculated from the linear regression analysis (Fig. 5) that the ED_{50} was 6.1 mg/kg for an intensity of stimulation 3 × threshold for C-fiber activation.

4. Discussion

The response characteristics of the spinal trigeminal nucleus oralis convergent neurons recorded in this experiment are in general agreement with data from former studies (Dallel et al., 1990; Raboisson et al., 1995). All the neurons we tested had a mechanoreceptive field involving one or two trigeminal divisions and were shown to receive C- as well as A-fiber afferent inputs. The receptive field frequently included oral or perioral regions. Interestingly, the C-fiber-evoked responses were qualitatively and quantitatively similar to their counterparts recorded from neurons within the spinal trigeminal nucleus caudalis (Sp5C) (Villanueva and Le Bars, 1985; Bouhassira et al., 1987; Raboisson et al., 1995).

The present results demonstrated that systemic morphine depresses C-fiber-evoked activities of spinal trigeminal nucleus oralis neurons, in a dose-dependent and naloxone-reversible fashion. The depressive effect of morphine was nearly maximal 5 min after the injection, since no further significant increase had been observed 10 min later. The latency of appearance of these effects is consistent with results from numerous previous studies regarding various central nervous system levels with i.v. administration of morphine (Le Bars et al., 1979, 1980; Woolf and Fitzgerald, 1981; Hylden and Wilcox, 1986; Bing et al., 1989; Huang et al., 1993).

The effects of morphine on C-fiber-evoked responses in the spinal trigeminal nucleus oralis were similar to those obtained in the deep dorsal horn, under the same experimental conditions. Indeed, for this area the ED_{50} value of the responses to percutaneous activation of C-fibers (Le Bars et al., 1979, 1980) was 6.3 compared to 6.1 mg/kg in the spinal trigeminal nucleus oralis. The linear aspect of the dose-response curve and its reversal by the opiate antagonist, naloxone, strongly suggest that specific opiate receptors are involved in the observed effects.

The mechanism through which systemic morphine exerts its antinociceptive action in spinal trigeminal nucleus oralis is unknown but three hypotheses can be suggested to explain these effects. First, morphine may act directly at the level of the spinal trigeminal nucleus oralis. However, the absence of opioid receptors (Atweh and Kuhar, 1977) does not support this idea. In addition, Andersen et al. (1977) have demonstrated that morphine administered microelectrophoretically near the somata of spinal trigeminal nucleus oralis neurons, does not generally affect their spontaneous firing. However, a direct action of morphine on the spinal trigeminal nucleus oralis is suggested by results of behavioral studies where microinjection of morphine into the spinal trigeminal nucleus oralis depressed the nociceptive behavior induced by a subcutaneous injection of formalin in the upper lip (Luccarini et al., 1995) or significantly elevated the latency to a defensive face-rub reaction in response to noxious facial heat (Rosenfeld et al., 1983). Further studies are necessary to confirm this hypothesis.

A second mechanism can be proposed in which morphine, by an indirect action on some brainstem structures, mainly around the periaqueductal gray matter, would reinforce descending inhibitory controls which act on trigeminal nociceptive transmission. Nevertheless, conflicting results have been reported with regard to the effects of morphine microinjected into the periaqueductal gray or nucleus raphe magnus, on noxious-evoked dorsal horn neuronal activity. Some investigators have reported that morphine inhibits dorsal horn neurons responses to noxious thermal stimulation (Gebhart and Jones, 1988). Conversely, Le Bars et al. (1987), have shown that morphine microinjected into the same regions either had no effect or facilitated C-fiber-evoked activity of dorsal horn neurons. Le Bars et al. (1987) proposed that morphine does not increase the descending inhibitory influence originating from the periaqueductal grey or nucleus raphe magnus and acting on the spinal nociceptive transmission. Other electrophysiological data support this hypothesis, for example, in intact and spinal rats the depressive effects of i.v. morphine on C-fiber-evoked responses were found to be remarkably similar (Le Bars et al., 1980). Since, in our study, the ED_{50} is similar to those noted for deep dorsal horn cells in both intact and spinal rats (Le Bars et al., 1980, 1987), it seems unlikely that the increase of the descending inhibitory controls accounts for this effect.

According to the third hypothesis morphine would depress spinal trigeminal nucleus oralis neuron activity via an action through the Sp5C. Electrophysiological studies have shown that the Sp5C exerts a tonic facilitation on spinal trigeminal nucleus oralis neurons (Khayyat et al., 1975; Greenwood and Sessle, 1976; Hu and Sessle, 1979). The proposed anatomical substrate for this effect was the intranuclear trigeminal pathway (Falls, 1984; Ikeda et al., 1984; Nasution and Shigenaga, 1987). Depression of the facilitatory influence could explain the inhibitory effects

seen after morphine injection in our study. This idea is supported by results of autoradiographic binding and immunohistochemical studies which have demonstrated that in the trigeminal sensory complex, opiate receptors and enkephalinergic neurons are mainly located in the substantia gelatinosa of the Sp5C (Hökfelt et al., 1977; Sar et al., 1978; Finley et al., 1981; Sumal et al., 1982; Khachaturian et al., 1983; Murakami et al., 1987). In addition, previous works on the spinal dorsal horn (see Duggan and North, 1984) has shown that the site of action of morphine is situated in the substantia gelatinosa: following recording of convergent neurons the authors observed that morphine ejected near the cell body of convergent neurons of deep laminae had little effect but that ejection into the substantia gelatinosa induced inhibition of the neuronal responses to noxious stimuli. Thus, a similar mechanism of action could be proposed for spinal trigeminal nucleus oralis convergent neurons.

In conclusion, the present study demonstrated that morphine depresses the C-fiber-evoked responses of spinal trigeminal nucleus oralis convergent neurons in a dose-related (3–12 mg/kg range) and naloxone-reversible fashion. This effect is consistent with our statement, based on electrophysiological studies, that the spinal trigeminal nucleus oralis plays an important role in the transmission of trigeminal nociceptive information. The site where morphine exerts its antinociceptive action on spinal trigeminal nucleus oralis convergent neurons is unknown and further studies are required to clarify the point. However, it may be suggested that morphine could inhibit the tonic facilitation exerted by Sp5C on spinal trigeminal nucleus oralis neurons.

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