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OPIOID ACTIONS ON SINGLE NUCLEUS RAPHE MAGNUS NEURONS FROM RAT AND GUINEA-PIG *IN VITRO*

BY Z. Z. PAN, J. T. WILLIAMS AND P. B. OSBORNE

From the Vollum Institute, Oregon Health Sciences University, 3181 SW Sam Jackson Park Road, Portland, OR 97201, USA

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SUMMARY

1. Intracellular recordings were made from neurons of the nucleus raphe magnus (NRM) from rat ($n = 128$) and guinea-pig ($n = 115$). Two types of cells were found in each, primary (103 in rat, 27 in guinea-pig) and secondary cells (25 in rat, 88 in guinea-pig).

2. Primary cells had input resistances of 186 ± 9 MΩ ($n = 9$) in rat and 255 ± 50 MΩ ($n = 11$) in guinea-pig. The action potential in each was about 1·5 ms in duration. Synaptic potentials were evoked by focal electrical stimulation and consisted of both γ-aminobutyric acid (GABA) and excitatory amino acid components.

3. Morphine, [Met^5]enkephalin (ME) and [D-Ala^2, *N*-Me-Phe^4, Gly^5-ol]enkephalin (DAMGO) depressed the amplitude of the GABA-mediated synaptic potential by a maximum of 50–65% and had little effect on the excitatory amino acid-mediated synaptic potential. There was no effect of these opioids on the resting membrane potential or input resistance of primary cells in rat or guinea-pig.

4. Secondary cells had short duration action potentials (< 1 ms) and an input resistance of 354 ± 47 MΩ in rat ($n = 6$) and 290 ± 40 MΩ in guinea-pig ($n = 15$). The synaptic potential observed in the cells of this group was mediated by activation of only excitatory amino acid receptors.

5. ME hyperpolarized and/or abolished the spontaneous firing in sixteen out of twenty-four neurons in the secondary group from rat and eighty out of eighty-four neurons from guinea-pig. ME induced an outward current at −60 mV that reversed polarity at potentials more negative than -92 ± 3 mV in rat ($n = 6$) and -98 ± 2 mV in guinea-pig ($n = 18$). The reversal potential of the opioid current was shifted to less negative potentials when the external potassium concentration was increased, as predicted by the Nernst equation.

6. The morphology of the two types of cells were distinguishable in that primary cells were oval (29×18 μm in rat; 36×19 μm in guinea-pig) with two to four thick tapering dendrites that branched within 50 μm of the cell body. Secondary cells were generally round or oval (about 24×13 μm in rat; 27×17 μm in guinea-pig) with two to five thin non-tapering dendrites.

7. The results suggest that opioids increase the activity of a population of NRM neurons by presynaptic depression of GABA-mediated inhibitory input. This could

be one of the mechanisms by which opioids modulate the descending inhibition from the NRM in the endogenous pain-modulating system.

INTRODUCTION

Nucleus raphe magnus (NRM), located in the rostral medulla, has received much attention because of the involvement of its spinal projection in the endogenous pain-modulating system of the brain (Mayer & Price, 1976; Basbaum & Fields, 1984). Previous studies in rat and cat have shown that antinociception can be produced when the neurons in the NRM are activated. For example, stimulation in the NRM inhibits spinal nociceptive neurons which were activated by noxious stimuli (Duggan & Griersmith, 1979; Giesler, Gerhart, Yezierski, Wilcox & Willis, 1981; Gebhart, 1986; Light, Casale & Menetrey, 1986). Stimulation in the periaqueductal grey (PAG) caused inhibition of spinal nociceptive neurons and excited neurons in the NRM (Behbehani & Fields, 1979; Mason, Strassman & Maciewicz, 1988). The descending inhibitory projection of the NRM is also involved in opioid-induced analgesia, for example, damage to the NRM or its spinal projection significantly reduces the antinociception produced by systemic opioids or micro-application of opioids into the PAG or the NRM (Basbaum & Fields, 1984).

One major component of the descending projection from the NRM is inhibitory, suggesting that opioids produce analgesia by activating NRM neurons. Many NRM neurons, including these projecting to the spinal cord, were excited by systemic administration or microinjection of opioids to the PAG (Anderson, Basbaum & Fields, 1977; Fields & Anderson, 1978). It should be noted that most studies also report that a significant proportion of NRM cells were inhibited by opioids (Deakin, Dickenson & Dostrovsky, 1977; Fields & Anderson, 1978; Toda, 1982; Duggan & North, 1983; Chiang & Pan, 1985). The role that these neurons play in descending pain modulation and opioid analgesia is not known.

Little is known about the mechanism by which opioids activate some NRM neurons and inhibit others (Duggan & North, 1983; Basbaum & Fields, 1984). In the present study, the membrane properties and responses to opioids of NRM neurons were studied in the slice preparation. An abstract of some of this work has been presented (Pan & Williams, 1989*a*).

METHODS

Intracellular recordings were made from NRM cells in slices of rat and guinea-pig medulla. The methods employed were similar to those published previously for the dorsal raphe (Pan & Williams, 1989*b*). Briefly, brain slices (300 μm) were cut in a vibratome in cold (4 °C) physiological saline. Slices (coronal) were taken from the level of the facial nerve where the facial nucleus was largest. A single slice was placed in a tissue bath through which flowed physiological saline (1·5 ml/min) at 37 °C. The content of the physiological saline solution was (mM): NaCl, 126; KCl, 2·5; NaH_2PO_4, 1·2; $MgCl_2$, 1·2; $CaCl_2$, 2·4; glucose, 11; $NaHCO_3$, 25; gassed with 95% O_2 and 5% CO_2 at 37 °C.

The area of the NRM was recognized in the slice as a triangular area in the mid-line just above the pyramidal tracts. Neurons were penetrated with glass microelectrodes filled with potassium chloride (2 M) having a resistance of 40–80 MΩ. Membrane currents were recorded with a single-electrode voltage-clamp amplifier (Axoclamp 2A) using switching frequencies between 3 and 6 kHz. The setting time of the clamp following a 10 mV step was typically 3–5 ms. Steady-state current–voltage (I–V) plots were constructed directly on an x–y plotter using a slow

depolarizing ramp potential. The speed of the ramp (1 mV/s) was sufficiently slow to give the same current as that measured at the termination of a 2 s step.

Bipolar tungsten stimulating electrodes were placed in the slice, lateral (100–300 μm) and dorsal (100–300 μm) to the NRM. Single electrical stimuli (0·05–0·4 ms) of constant voltage were used to evoke synaptic potentials. Such local stimulation was expected to activate all the fibres in this area. Placement of the stimulating electrode and adjustment of the stimulus voltage were made so that no antidromic or directly activated action potentials were evoked.

Drugs were applied by superfusion. The following drugs and salts were used: 6-cyano-2,3-dihydroxy-7-nitro-quinoxaline (CNQX, Tocris Neuramin), DL-2-amino-5-phosphonovaleric acid (APV), kynurenic acid, (−)-bicuculline methiodide, [Met^5]enkephalin (ME), [D-Ala^2,*N*-Me-Phe^4,Gly^5-ol]enkephalin (DAMGO), [D-Pen^2,D-Pen^5]enkephalin (DPDPE), γ-aminobutyric acid (GABA) (all from Sigma) and naloxone (Endo). Numerical data are presented as means ± S.E.M. Comparisons of experimental data were carried out using a paired *t* test.

In some experiments, the recording electrode was filled with a solution containing KCl (2 mM), Tris (50 mM, pH = 7·2) and biocytin (2%, Sigma). Usually diffusion of biocytin alone was sufficient to fill the cell although sometimes hyperpolarizing current (20–60 pA) was used to speed diffusion (Horikawa & Armstrong, 1988). Recordings for at least 20 min were required to obtain sufficient staining. Only one cell was labelled in each slice. The properties of the electrodes, the cells and the responses to drugs using this electrode solution were not distinguishable from KCl (2 M)-filled electrodes. After the experiment, the slice was fixed (2% formaldehyde/15% picric acid in 0·1 M-phosphate buffer, pH 7·0) overnight and then washed successively in ethyl alcohol (80%), dimethyl sulphoxide (DMSO) and three times in phosphate buffer solution (PBS, 0·1 M, pH 7·2, 15–20 min each). The slice was then incubated with fluorescein isothiocyanate conjugated streptavidin (1:300, Jackson Immunoresearch Laboratory) overnight in a humid chamber and washed with PBS three times. The slices were mounted in buffered glycerol and examined using a fluorescence microscope.

RESULTS

Two types of cells in rat

Intracellular recordings were made from a total of 128 cells in rat. Two types of cells were distinguished, 103 of the 128 cells were called primary cells and the other 25 cells were termed secondary cells.

Primary cells

The cells in this group had resting membrane potentials between −50 and −75 mV and were generally not spontaneously active (8 out of 104 fired action potentials spontaneously). The input resistance measured near the resting membrane potential was 186 ± 9 MΩ (range 143–235 MΩ, $n = 14$). Action potential duration measured at the threshold for activation was between 1–1·8 ms (Fig. 1*A*).

Focal electrical stimulation evoked a synaptic potential with a latency of 0·5–3 ms that peaked within 4–10 ms and had a total duration less than 200 ms. The amplitude of the depolarization was dependent on the stimulation intensity and action potentials could be evoked at the peak of larger depolarization. All cells were hyperpolarized by passing current through the recording electrode to hold the membrane potential at about −70 mV in order to obtain a 10–15 mV depolarization without activating action potentials. This synaptic potential was completely blocked by tetrodotoxin (TTX, 1 μM), $CoCl_2$ (2 mM) or solutions containing no calcium.

In all cells tested ($n = 61$) in this primary group, the synaptic potential induced by the stimulation was depressed in amplitude by excitatory amino acid receptor antagonists (APV, 10 μM and CNQX, 10 μM). The amplitude of the synaptic potential that remained was 73 ± 7% ($n = 8$) of the total depolarizing synaptic

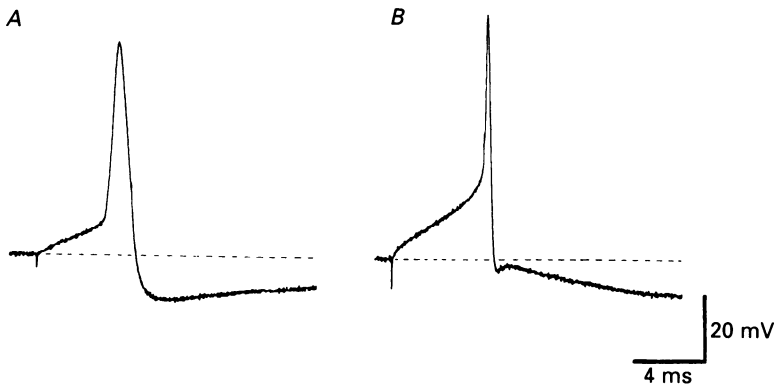

Fig. 1. Action potentials from two cell types in rats. *A*, a primary cell and *B*, a secondary cell. Depolarizing electrotonic potentials were applied to evoke the action potentials from −65 mV.

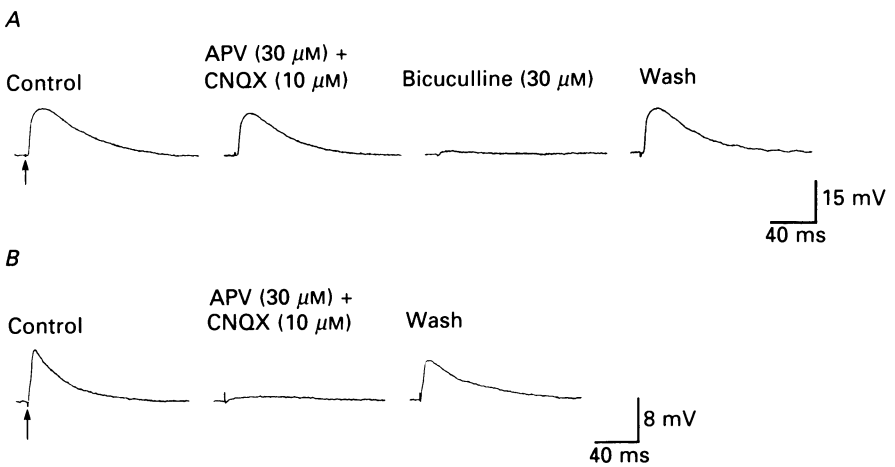

Fig. 2. Separation of the GABA and excitatory amino acid synaptic potentials in two types of cells from rats. KCl-filled electrodes were used in each. *A*, a primary cell where a small reduction in the synaptic potential amplitude was produced by superfusion with APV and CNQX. Addition of bicuculline to the solution containing APV and CNQX completely blocked the remaining synaptic potential. *B*, a secondary cell. The synaptic potential was almost completely abolished by APV and CNQX. Each trace is the average of four. Small arrows indicate the stimulus artifact.

potential (Fig. 2*A*). That synaptic potential had a slower rate of rise and was completely blocked by bicuculline (30 μM, Fig. 2*A*). This $GABA_A$-mediated synaptic potential was depolarizing (from −70 mV) in these experiments because of the use of KCl-filled electrodes. The reversal potential of $GABA_A$ receptor-mediated current induced by muscimol (1–3 μM) was -46 ± 3 mV ($n = 5$).

The amplitude of the excitatory amino acid component, studied in the presence of bicuculline (30 μM), was generally smaller than the GABA component when the same stimulus strength was employed ($46 \pm 5\%$ of the total synaptic potential, $n = 5$).

Secondary cells

The resting membrane potential in this group of cells was between −50 and −68 mV with input resistance of 354±47 MΩ (range: 200–571 MΩ, $n = 6$) and the action potential duration was < 1 ms (Fig. 1*B*). Spontaneous action potentials were recorded in seven of the twenty-five cells. The synaptic potentials were completely blocked by APV (10 μM) and CNQX (10 μM) in all cells tested ($n = 10$). Bicuculline (30 μM) had no effect on the synaptic potential in these cells. Unlike the primary cell group, these cells appeared to receive mostly excitatory amino acid input (Fig. 2*B*).

Two types of cells in guinea-pig

In guinea-pig, 27 of 115 cells were the primary cell type and 88 cells were the secondary type. Unlike those in rat, about 70% of all cells (both primary and secondary) fired action potentials spontaneously.

Primary cells

The primary cells in guinea-pig had characteristics similar to those of primary cells in rats. That is, the action potential was > 1 ms in duration and there were synaptic potentials mediated by both GABA and an excitatory amino acid. The 'resting' membrane potential varied from −52 to −70 mV and the input resistance was 255±50 MΩ (range: 105–660 MΩ, $n = 11$).

Secondary cells

Secondary cells in the guinea-pig were similar to those found in the rat in that they had action potentials of shorter duration (< 1 ms) and a fast synaptic potential that was mediated by an excitatory amino acid. The input resistance of these cells was 290±40 MΩ (range: 118–667 MΩ, $n = 15$). The amplitude of synaptic potentials was 8·7±0·4 mV in control, 10·9±0·1 mV in bicuculline (30 μM) and 0·7±0·2 mV in bicuculline plus APV (30 μM) and CNQX (10 μM), ($n = 4$).

Actions of opioids in rat

Primary cells

The synaptic potential was depressed by opioids. The actions of opioids on the excitatory amino acid and $GABA_A$ synaptic potentials were studied separately. No change in the resting membrane potential or input resistance was caused by ME, DAMGO or DPDPE in these cells including those that were firing spontaneously.

$GABA_A$ synaptic potential. These experiments were carried out in the presence of CNQX (10 μM) and APV (10 μM) or kynurenic acid (500 μM). ME, DAMGO and morphine depressed the amplitude of the GABA-mediated synaptic potential in every cell tested (Fig. 3*A*). The inhibition induced by ME was dependent on the concentration applied having a threshold of 100 nM and an EC_{50} of about 1 μM. The maximum inhibition was about 50% of the control at 30 μM (Fig. 3*B*).

The selective μ-opioid receptor agonist, DAMGO, caused concentration-dependent inhibition of the GABA-mediated synaptic potential (Fig. 4*A*). The threshold concentration was 10 nM, with an EC_{50} of about 70 nM and the maximum inhibition (at 1 μM) was 58±6% ($n = 5$). The concentration–response curve for DAMGO was

shifted to the right in the presence of naloxone (100 nM, Fig. 4*B*). The EC_{50} for DAMGO in naloxone was 4·8 μM which was about 65-fold larger than that in the control. The estimated naloxone K_d from this single shift (Kosterlitz & Watt, 1968) was 1·5 nM. DPDPE (300 nM–1 μM), a selective δ-opioid receptor agonist, had no

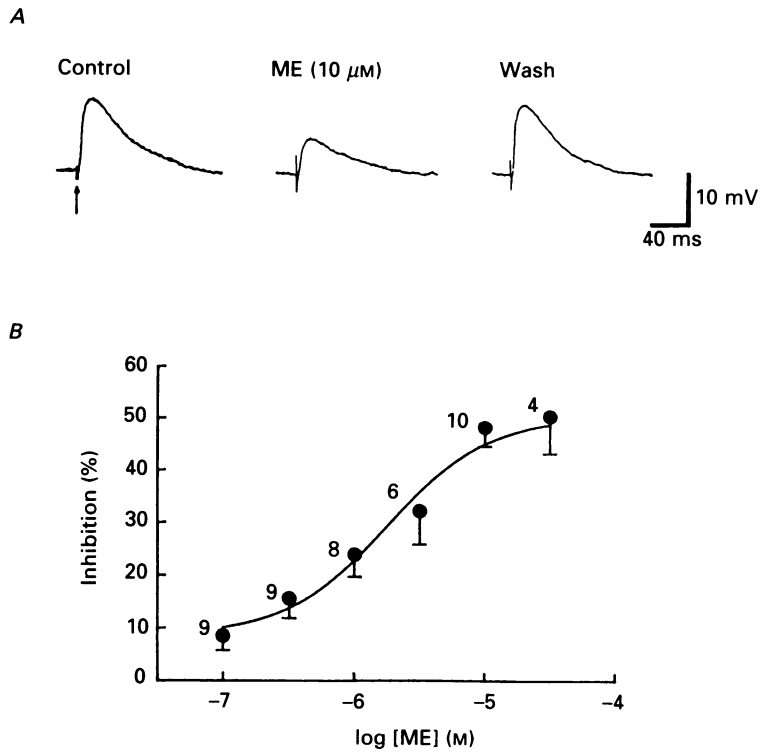

Fig. 3. Opioids inhibit the GABA-mediated synaptic potential in rat primary cells. *A*, GABA-mediated synaptic potential (average of four) in control (left), in the presence of ME (10 μM) (middle) and after wash-out (right). No change in the resting membrane potential was produced by ME. *B*, concentration dependence of the ME-induced inhibition of GABA-mediated synaptic potentials. Percentage inhibition of GABA synaptic potential plotted as a function of the ME concentration. Numbers beside each point indicate the number of cells tested at that concentration. The amplitude of the GABA synaptic potential in control was 13·4 ± 1·0 mV ($n = 10$). All these experiments were carried out in the presence of APV and CNQX to block excitatory amino acid receptors.

effect on the GABA-mediated synaptic potential (control = 12·3 ± 0·6 mV, DPDPE (300 nM) = 12·1 ± 0·8 mV; $n = 6$; $P > 0·05$).

Excitatory amino acid synaptic potential. The excitatory amino acid-mediated synaptic potential was studied in the presence of bicuculline (30 μM). The average amplitude was 7·6 ± 1·1 mV ($n = 7$) in control and 6·7 ± 1·1 mV ($n = 7$) in the presence of ME (10 μM, Fig. 5*A*). The small inhibition of the synaptic potential, a reduction to 90 ± 2% of the control ($n = 7$), was significant ($P < 0·02$) but did not recover after wash-out.

GABA applied exogenously. The depolarization produced by GABA applied by superfusion was not affected by either ME (10 μM) or DAMGO (300 nM) in each of five

cells tested (Fig. 5*B*). Superfusion with bicuculline (30 μM) blocked most of the GABA-induced depolarization.

Secondary cells

ME (10 μM) or DAMGO (300 nM) caused a hyperpolarization or inhibition of sixteen out of twenty-four secondary cells tested. The membrane potential of the

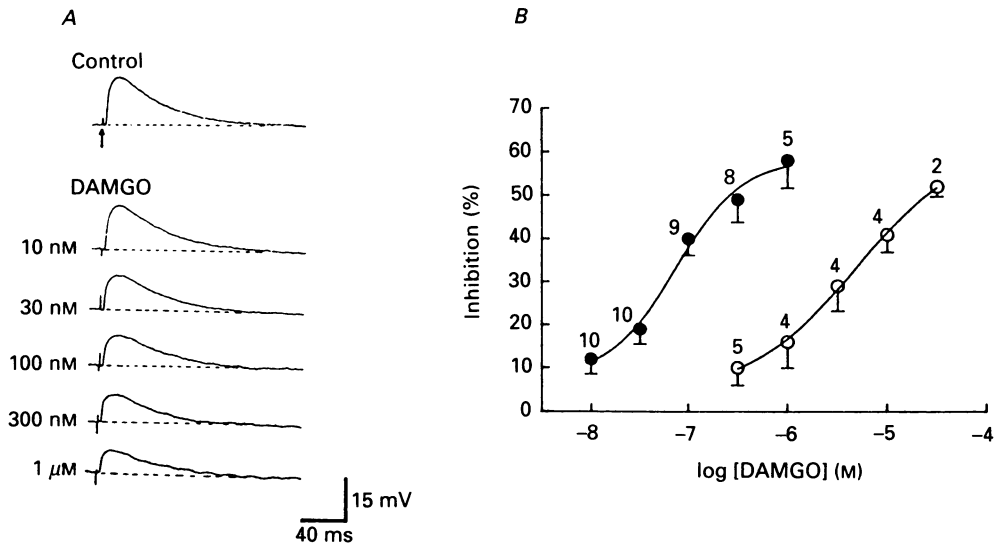

Fig. 4. Concentration-dependent inhibition of GABA-mediated synaptic potentials by DAMGO in primary cells from rats. Experiments were carried out after blockade of excitatory amino acid receptors with APV and CNQX. *A*, DAMGO (10 nM–1 μM) caused an inhibition of the GABA-mediated synaptic potential. Each trace is the average of four. *B*, concentration-dependence curve of the percentage inhibition of the GABA synaptic potential as a function of the DAMGO concentration, in control (●) and in the presence of naloxone (100 nM) (○). The EC_{50} for DAMGO was 74 nM in control and 4·8 μM in naloxone.

remaining cells was not affected by opioids. ME (10 μM) caused a 6 ± 1 mV ($n = 12$) hyperpolarization of secondary cells (Fig. 6A). The spontaneous firing of the other four neurons was completely blocked. Under voltage-clamp, ME (10 μM) caused an outward current that reversed polarity at -92 ± 3 mV ($n = 6$). In a single experiment the reversal potential of the ME-induced current was shifted to -85 and -70 mV as the external potassium was increased to 6·5 and 10·5 mM, respectively. None of the opioids tested produced a change in the excitatory amino acid-mediated synaptic potential in these cells.

Effects of opioids in guinea-pig

Primary cells

As was found in rat, opioids, had no effect on the membrane potential and the GABA-mediated synaptic potential was depressed. The amplitude of the GABA synaptic potential was $12{\cdot}0 \pm 1{\cdot}4$ mV in control and $5{\cdot}1 \pm 0{\cdot}6$ mV in the presence of DAMGO (300 nM, $n = 8$), whereas DPDPE was without effect ($7{\cdot}8 \pm 0{\cdot}3$ in control;

7·6±0·2 in DPDPE, 300 nM). As in the rat, DAMGO (300 nM) also caused a small reduction in the amplitude of the excitatory amino acid-mediated synaptic potential (from 8·7±1·1 mV to 7·5±1·1 mV, $n = 4$).

Secondary cells

In eighty of eighty-four secondary cells ME (10 μM) caused a hyperpolarization (Fig. 6*B*). The amplitude of the hyperpolarization induced by ME (10 μM) was

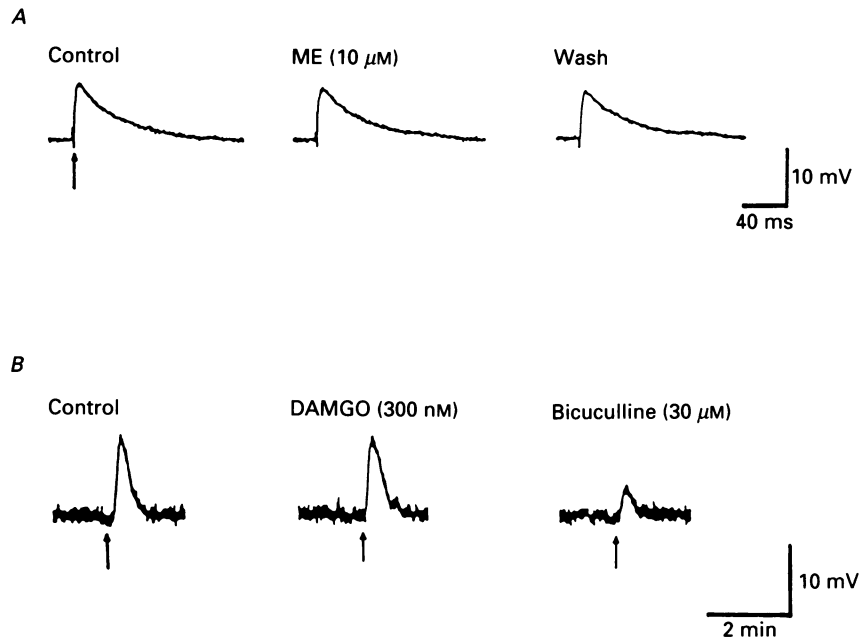

Fig. 5. Opioids had little effect on the excitatory amino acid-mediated synaptic potential and the depolarization induced by exogenously applied GABA in rat primary cells. *A*, recordings from a primary cell in the presence of bicuculline (30 μM) to block the $GABA_A$ receptors. Excitatory amino acid-mediated synaptic potentials (average of four) before (left), during (middle) and after (right) application of ME (10 μM). The resting membrane potential was not changed by ME. *B*, depolarization induced by exogenously applied GABA was not changed by DAMGO. Left: superfusion (indicated by the arrow) with GABA (10 mM) for 4 s produced a depolarization. Middle: DAMGO (300 nM) had no effect on the GABA-mediated depolarization. Right: the GABA-mediated depolarization was reduced by bicuculline (30 μM).

9±1 mV ($n = 64$), DAMGO (300 nM) was 10±1 mV ($n = 15$) and DPDPE (300 nM) produced a 3±1 mV hyperpolarization ($n = 12$).

Under voltage-clamp (at −60 mV), an outward current was induced by ME or DAMGO with an increase in conductance. The current reversed polarity at −103±0·7 mV ($n = 7$) in a solution with normal potassium concentration. As the potassium concentration in the perfusing solution was increased to 6·5 mM and 10·5 mM, the reversal potential shifted to −80±3 mV and −67±4 mV, respectively

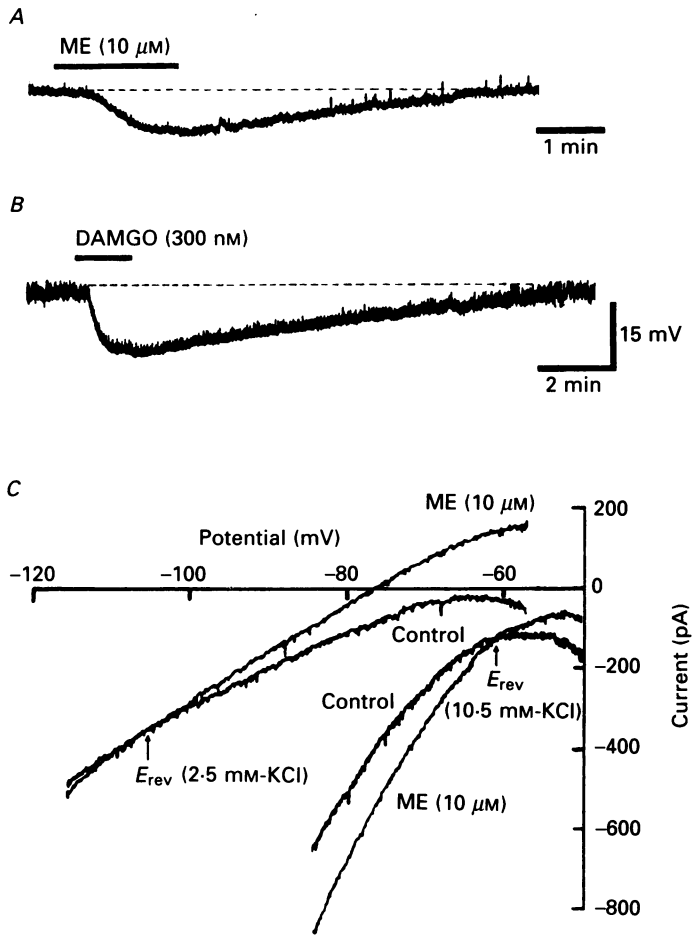

Fig. 6. Opioids hyperpolarized secondary cells by increasing potassium conductance. *A*, recording of membrane potential from a secondary cell in the rat. ME (10 μM) produced a hyperpolarization of 9 mV from −62 mV. *B*, in the guinea-pig, DAMGO (300 nM) caused a 12 mV hyperpolarization from −70 mV (this cell was held at −70 mV by passing negative current through recording electrode). *C*, steady-state current–voltage plots in the presence and absence of ME (10 μM) in two different concentrations of potassium from another secondary cell in the guinea-pig. The reversal potential was shifted from −105 mV in 2·5 mM potassium to −62 mV in 10·5 mM potassium.

($n = 7$, Fig. 6*C*). These shifts in reversal potential with extracellular potassium concentration were in accordance with the relationship predicted by the Nernst equation (slope $= -58{\cdot}5 \pm 1{\cdot}2$, $n = 7$).

Intracellular staining

The cellular morphology of raphe magnus neurons has been described in rat (Steinbusch & Nieuwenhuys, 1983), rabbit (Felten & Cummings, 1979), and cat (Edwards, Johnston, Poletti & Foote, 1987). In rat, two basic cell types could be distinguished: medium-sized cells (approximately 21 μm long) that were multipolar and oval in shape, and large cells (approximately 35 μm long) that were fusiform. In

the present study, twenty electrophysiologically characterized cells in rat (twelve primary and eight secondary) and fifteen cells in guinea-pig (nine primary and six secondary) were filled with biocytin and subsequently visualized.

Primary cells

In rat these cells had an average length of 29 μm (range: 22–47 μm) and width of 18 μm (range: 13–28 μm). Most cells were oval (9 of 12), two others were bipolar and

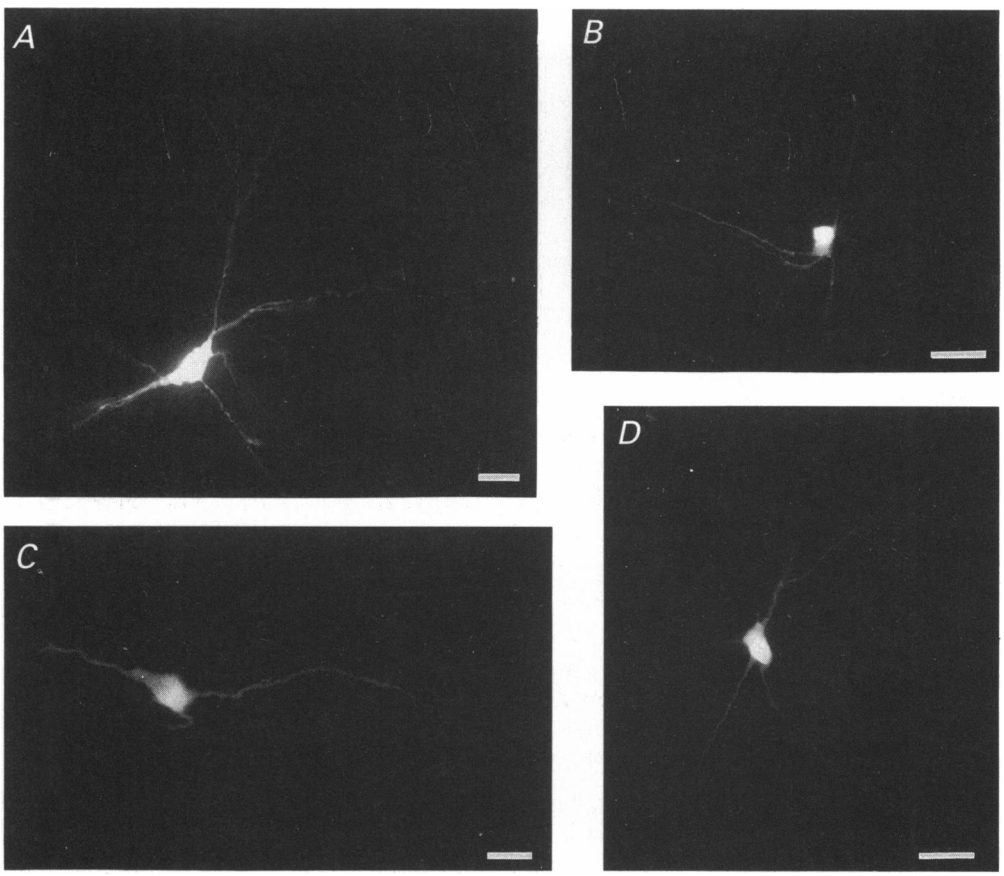

Fig. 7. Representative NRM neurons filled with biocytin and labelled with FITC-conjugated streptavidin. *A*, rat primary cell. *B*, rat secondary cell. *C*, guinea-pig primary cell. *D*, guinea-pig secondary cell. Scale bars = 30 μm.

one was tripolar. Most cells had two to four primary dendrites that were large in diameter near the cell body and tapered rapidly within 20–40 μm of the cell body. These dendrites branched within 20–50 μm of the cell body giving rise to multiple secondary dendrites (Fig. 7*A*). The dendritic tree of most cells tended to be orientated in the medio-lateral direction, this was most strongly evident for the bipolar cells.

In guinea-pig, nine primary cells were stained. These cells were larger than those

in rat having an average length of 36 μm (range: 22–23 μm) and width of 19 μm (range: 16–27 μm) and were very similar in morphology to the rat primary cells (Fig. 7*C*).

Secondary cells

Secondary cells in the rat tended to be smaller than primary cells with an average length of 24 μm (range: 17–31 μm) and width of 13 μm (range: 11–19 μm). All eight cells were round or oval in shape and had three to five primary dendrites (Fig. 7*B*). In these cells the primary dendrites did not taper in diameter within 20–40 μm of the cell body as was observed in the primary cells. Few secondary dendrites were seen to arise from the primary dendrites within 50–100 μm of the cell body. The processes did not appear to have any particular orientation.

In guinea-pig, all six cells were round or oval with an average length of 27 μm (range: 20–38 μm) and width of 17 μm (range: 13–19 μm) (Fig. 7*D*). These cells like those observed in rat had two to four primary dendrites with little or no taper in the diameter as they left the cell body. Unlike the rat, numerous secondary dendrites were seen within 30–60 μm of the cell body.

DISCUSSION

Two cell types

Two distinct types of NRM cells were found in both rat and guinea-pig. In rat, the primary cell group made up the major population of neurons. The major differences between the two populations were in the type of synaptic potentials and the response to opioids. In primary cells there was a large GABA-mediated synaptic potential, whereas little or no GABA-mediated synaptic potential was found in secondary cells. In both cell types, excitatory amino acid-mediated synaptic potentials were observed. The membrane potential of primary cells was not significantly changed by opioids, while the spontaneous activity of most secondary cells was inhibited and/or the membrane potential was hyperpolarized by opioids.

Differences in morphology were also noted. Primary cells tended to be larger and showed extensive dendritic branching in the region of the cell body. Secondary cells seemed smaller than primary cells and had few secondary dendrites near the cell body (in rat). The major difference between these cell types was the shape of the primary dendrites as they left the cell soma. The dendrites of primary cells arose from thick extensions of the cell soma whereas no such thickenings of the soma were observed in secondary cells.

One species difference was the relative abundance of secondary cells in the guinea-pig, although this difference may be a sampling error resulting from the use of microelectrodes. Another species difference was the morphology: both types of cells were larger in guinea-pig and a greater number of dendritic branches were found near the cell soma of secondary cells in guinea-pig. The difference in size may account for the increase in the number of recordings from guinea-pig secondary cells. In any case, the two cell types in each species seem similar in all other respects and suggest that these two cell types could be functionally common cell populations. Correlations have been made between NRM cell types from rat and cat in control of descending

pain modulation (Fields, Barbaro & Heinricher, 1988), but no similar studies have been made in guinea-pig.

Actions of opioids in primary cells

In the rat and guinea-pig, opioids inhibit the GABA-mediated synaptic potential by about 50% in the primary cells of the NRM. DAMGO, a selective μ-opioid receptor agonist, was effective, whereas DPDPE, a selective δ-opioid receptor agonist, was not. In addition, in rat, the K_d for naloxone was estimated to be about 1·5 nM suggesting an action on a μ-opioid receptor. These findings suggest that the opioid-induced inhibition of the GABA-mediated synaptic potential was mediated by a μ-opioid receptor. Previous autoradiographic studies have described μ-opioid binding in the NRM of rat (Bowker & Dilts, 1988; Mansour, Khachaturian, Lewis, Akil & Watson, 1988). Since neither the resting membrane potential of the cell nor the response of the cell to exogenously applied GABA was changed by ME or DAMGO, the inhibition of the GABA-mediated synaptic potential by opioids probably results from presynaptic inhibition of GABA release.

The GABA-mediated synaptic potential was the predominant 'fast' synaptic potential in primary neurons of the NRM under the conditions of these experiments. It has been shown in the dorsal raphe nucleus that the GABA-mediated synaptic potential was inhibitory at resting membrane potentials when non-chloride electrodes were used and reversed its polarity at about -70 mV (Pan & Williams, 1989*b*). Moreover, exogenously applied GABA caused an inhibition of NRM neurons *in vivo* (Nishikawa & Scatton, 1985). Therefore, opioids would be expected to increase the activity of primary neurons by disinhibition. Some of these primary neurons may project to the dorsal horn of the spinal cord. Consequently, descending inhibition from the NRM could be facilitated by opioids. This may be one of the mechanisms involved in the opioid-produced antinociception.

Two physiologically identified group of cells have been described in the NRM *in vivo* based on their response to a noxious stimulus, 'off' and 'on' cells (Fields, Vanegas, Hentall & Zorman, 1983; Fields *et al.* 1988). In response to a noxious stimulus, the firing of 'off' cells ceased just prior to the tail flick. Opioids prevented this inhibition in firing. 'On' cells were defined by a burst of firing just before the tail flick in response to a noxious stimulus. Opioids blocked this burst of activity. The effect of opioids on the activity of primary cells described here correlates with 'off' cells. In fact, excitation of 'off' cells by opioids was hypothesized to result from disinhibition because of the lack of convincing evidence for a direct excitatory opioid effect. The cells that were excited by opioids were thought to cause descending inhibition of spinal nociceptive dorsal horn neurons (Fields *et al.* 1988).

Actions of opioids in secondary cells

ME or DAMGO hyperpolarized most secondary neurons. DPDPE (300 nM) had no effect in the rat and only weakly hyperpolarized neurons in the guinea-pig. The results suggest that activation of μ-opioid receptors caused a hyperpolarization in both species, although in guinea-pig, a δ-opioid receptor-mediated hyperpolarization has not been ruled out. In each species, the hyperpolarization resulted from an increase in potassium conductance.

This group of cells is similar to the functionally defined 'on' cells (Fields *et al.* 1988)

in the response to opioids. These cells may function as local circuit interneurons (presumed to be GABA containing) or as a group of descending excitatory neurons which facilitate the nociceptive transmission in the spinal cord and are inhibited by opioids to cause analgesia (Fields *et al.* 1988).

Inhibition of local inhibitory neurons in the central nervous system has been suggested to be a common mechanism of opioid action (Nicoll, Alger & Jahr, 1980). Recently, interneurons that release GABA onto hippocampal pyramidal cells were found to be hyperpolarized by opioids (Madison & Nicoll, 1988). An inhibitory GABA input onto the NRM neurons involved in the regulation of nociceptive threshold has been suggested (Drower & Hammond, 1988). In addition, numerous GABA-transaminase-staining neurons have been identified immunohistochemically in the rat NRM (Nagai, Maeda, Imai, McGeer & McGeer, 1985). No direct morphology of those neurons was noted. A population of those (GABA-containing) neurons may be sensitive to opioids and thus mediate the presynaptic inhibition of GABA release onto primary cells.

In summary, we have found that the GABA-mediated synaptic potential in the primary cells of the NRM in rats and in guinea-pigs was reduced in amplitude by opioids acting on μ-opioid receptors. Reduction of this synaptic potential would be expected to increase activity of these neurons by disinhibition. In addition, many secondary neurons were directly inhibited by opioids through an increase in potassium conductance.

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