Opioid-Activated Postsynaptic, Inward Rectifying Potassium Currents in Whole Cell Recordings in Substantia Gelatinosa Neurons

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Schneider, S. P., W. A. Eckert III, and A. R. Light. Opioid-activated postsynaptic, inward rectifying potassium currents in whole cell recordings in substantia gelatinosa neurons. J. Neurophysiol. 80: 2954–2962, 1998. Using tight-seal, whole cell recordings from isolated transverse slices of hamster and rat spinal cord, we investigated the effects of the μ-opioid agonist (D-Ala²,N-Me-Phe⁴,Gly⁵-ol)-enkephalin (DAMGO) on the membrane potential and conductance of substantia gelatinosa (SG) neurons. We observed that bath application of 1–5 μM DAMGO caused a robust and repeatable hyperpolarization in membrane potential (ΔV m) and decrease in neuronal input resistance (R i) in 60% (27/45) of hamster neurons and 39% (9/23) of rat neurons, but significantly only when ATP (2 mM) and guanosine 5′-triphosphate (GTP; 100 μM) were included in the patch pipette internal solution. An ED₅₀ of 50 nM was observed for the hyperpolarization in rat SG neurons. Because G-protein mediation of opioid effects has been shown in other systems, we tested if the nucleotide requirement for opioid hyperpolarization in SG neurons was due to G-protein activation. GTP was replaced with the nonhydrolyzable GTP analogue guanosine 5′-O-(3-thiotriphosphate) (GTPγS; 100 μM), which enabled DAMGO to activate a nonreversible membrane hyperpolarization. Further, intracellular application of guanosine 5′-O-(2-thiodiphosphate) (GDPβS; 500 μM), which blocks G-protein activation, abolished the effects of DAMGO. We conclude that spinal SG neurons are particularly susceptible to dialysis of GTP by whole cell recording techniques. Moreover, the depletion of GTP leads to the inactivation of G-proteins that mediate μ-opioid activation of an inward-rectifying, potassium conductance in these neurons. These results explain the discrepancy between the opioid-activated hyperpolarization in SG neurons observed in previous sharp electrode experiments and the more recent failures to observe these effects with whole cell patch techniques.

INTRODUCTION

A strong presynaptic inhibitory mechanism has been postulated to play a major role in opioid analgesia at the spinal cord level. This presynaptic effect is mediated by decreasing transmitter release from sensory afferents (e.g., Chang et al. 1989; Duggan and North 1983; Glaum et al. 1994; Horii et al. 1992; Jessell and Iversen 1977; Suarez-Roca and Maixner 1992; Yaksh et al. 1988). Evidence from whole cell recordings of dorsal root ganglion cells suggests that the presynaptic actions of opioids are associated with the activation of intracellular pathways involving G-proteins, which modulate membrane conductance either directly (Moises et al. 1994; Wilding et al. 1995) or via second messengers, such as adenosine 3′,5′-cyclic monophosphate acting on voltage-gated calcium channels (Akins and McCleskey 1993; Chen et al. 1988; Ingram and Williams 1994; Reisine et al. 1996). However, the lack of morphological evidence of synaptic contacts onto primary afferent terminals and axons suggests that opioid receptors on primary afferents may function in a nonsynaptic manner (Ribeiro-da-Silva and Cuello 1995) and complicates the premise of a primarily presynaptic action of opioids on spinal neurons.

Previous in vitro studies with sharp microelectrode recordings from the spinal substantia gelatinosa (SG; Rexed’s lamina II), a main termination region for nociceptive afferent fibers, characterized direct postsynaptic effects of opioids on spinal neurons. These studies show that μ-opioid agonists activate a membrane hyperpolarization that is sensitive to the morphine antagonist naloxone in association with an increase in membrane conductance and a potassium-dependent outward current (Grudt and Williams 1994; Jefimina 1988; Murase et al. 1982; Yoshimura and North 1983). Whole cell patch-clamp recording studies of laminae I and II neurons demonstrated μ-opioid-receptor-mediated presynaptic inhibitory effects on excitatory synaptic currents; these studies, however, failed to document strong postsynaptic actions of μ-opioids on these same neurons (Glaum et al. 1994; Hori et al. 1992). Furthermore, recent reports suggested that opioids may modulate excitatory amino acid-mediated depolarization of trigeminal neurons via an opioid-selective, G-protein-coupled pathway (Kolaj and Randic 1996) or a nalo-oxone-insensitive pathway, possibly by interacting with sites on the N-methyl-D-aspartate (NMDA)-receptor-channel complex (Chen et al. 1995a,b), suggesting alternative inhibitory mechanisms as well.

In this study we document the effects of the μ-opioid agonist [D-Ala²,N-Me-Phe⁴,Gly⁵-ol]-enkephalin (DAMGO) on hamster and rat spinal SG neurons studied in vitro in the tight-seal, whole cell recording configuration. We found that DAMGO hyperpolarized ~60% of recorded hamster neurons and 39% of rat neurons and that this was associated with an outward current that was dependent on levels of extracellular potassium. However, strong hyperpolarization was observed only when G-protein coupling of receptor activation was maintained by including ATP and guanosine 5′-triphosphate (GTP) in the internal solution of the patch pipette, as suggested by Forscher and Oxford (1985). The DAMGO-evoked membrane hyperpolarization was markedly reduced when the nucleotide triphosphates ATP and GTP were omitted from the pipette internal solution and was affected by alterations in the phosphorylation of G-proteins, as produced by intracellular application of nonhydrolyzable analogs of guanosine nucleotides. This observation con-
firmed the susceptibility of opioid-receptor activation of membrane hyperpolarization to disruption of G-protein activation. Because earlier whole cell recording studies (Glaum et al. 1994; Hori et al. 1992) were done in the absence of GTP, we suggest that replenishment of intracellular nucleotide triphosphates is essential to observe robust opioid-activated postsynaptic membrane effects in spinal SG neurons. These results were published in preliminary form (Schneider and Light 1994a,b).

METHODS

Tissue preparation

All procedures involving the use of animals were approved by the IACUC of the University of North Carolina at Chapel Hill. After anesthesia with urethan (1.5 mg/g ip), 3- to 4-wk-old male Syrian hamsters (30–45 g) and 3- to 10-wk-old female Long-Evans rats (50–200 g) were cooled to 25°C. The vertebral column was rapidly removed and placed in a cold (3–8°C), oxygenated medium (Aghajanian and Rasmussen 1989) containing (in mM) 216 sucrose, 2.5 KCl, 2.0 CaCl₂, 2.0 MgSO₄, 1.25 NaH₂PO₄, 26 NaHCO₃, and 10 glucose, pH 7.35–7.45. A 5- to 6-mm block of lumbar spinal cord was excised, mounted to the stage of a vibratome 1000 (TPi, St. Louis, MO), and covered with cold dissection medium. Three to eight 500-μm-thick transverse sections were cut slowly (1–2 mm/ min) with a stainless steel razor blade at the highest vibration frequency available and collected in oxygenated artificial cerebrospinal fluid (ACSF) containing (in mM) 120 NaCl, 2.5 KCl, 2.5 CaCl₂, 26 NaHCO₃, 1.5 MgSO₄, 1.25 NaH₂PO₄, and 10 glucose, pH 7.35–7.45, 290–310 mosmol/l.

Electrophysiological recording

WHOLE CELL RECORDING. Individual slices were transferred to a glass-bottomed recording chamber (volume 1.5 ml) mounted on a fixed-stage microscope and superfused with ACSF (5–6 ml/min; 25°C). Recording pipettes were fabricated from borosilicate glass (N-51A, 1.5 mm tubing OD; Drummond Scientific, Broomall, PA) and filled with an internal solution (see Table 1); DC resistances were 6–10 MΩ. Pipettes were positioned within the SG, which was visible as a translucent band in the dorsal horn. Blind, tight-seal whole cell recordings from SG neurons (65–295 μm from the slice surface) were obtained as previously reported (Blanton et al. 1989; Yoshimura and Nishi 1993). Whole cell current and voltage signals were amplified (Axoclamp 1, 2A or Axopatch 1D; Axon Instruments, Foster City, CA), displayed on a storage oscilloscope, and analyzed on-line with pClamp or Axotape software (Axon Instruments) or saved on magnetic tape. A strip chart recorder monitored membrane potential (V_m) and neuronal input resistance (R_m). Measured values are expressed as means ± SD.

TABLE 1. Effect of recording pipette internal solution on resting membrane potential (V_m), neuronal input resistance (R_m), and spike amplitude for hamster neurons

<table>
<thead>
<tr>
<th>Internal Solution Constituents</th>
<th>V_m mV</th>
<th>R_m MΩ</th>
<th>Spike Amplitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 mM ATP/100 μM GTP</td>
<td>−54.2 ± 6.1 (22)</td>
<td>809 ± 291 (21)</td>
<td>79.2 ± 14.0 (26)</td>
</tr>
<tr>
<td>2 mM ATP</td>
<td>−54.7 ± 7.1 (11)</td>
<td>813 ± 353 (11)</td>
<td>82.0 ± 8.7 (3)</td>
</tr>
<tr>
<td>100 μM GTP</td>
<td>−53.9 ± 4.1 (7)</td>
<td>727 ± 177 (7)</td>
<td>75.0 ± 5.0 (3)</td>
</tr>
<tr>
<td>Basic</td>
<td>−55.4 ± 2.3 (8)</td>
<td>686 ± 170 (8)</td>
<td>77.7 ± 24.2 (3)</td>
</tr>
<tr>
<td>500 μM GDP/S</td>
<td>−55.6 ± 5.2 (23)</td>
<td>846 ± 353 (21)</td>
<td>78.8 ± 11.8 (4)</td>
</tr>
<tr>
<td>100 μM GTPy/S</td>
<td>−54.6 ± 4.9 (5)</td>
<td>838 ± 165 (4)</td>
<td>76 (1)</td>
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</tbody>
</table>

Values indicate means ± SD. GTP, guanosine 5’-triphosphate.

VOLTAGE CLAMP. Some experiments were performed in continuous or discontinuous single electrode voltage-clamp mode at holding potentials between −50 and −100 mV. Current-voltage plots from whole cell current responses were constructed by measuring steady-state membrane currents at the end of 1.0-s voltage steps from holding potentials of −55 or −60 mV. The liquid junction potential between the recording pipette and ACSF was determined to be −2 mV and was used to correct estimations of reversal potential for opioid-induced membrane responses. DAMGO-activated currents were determined by subtracting membrane currents in ACSF from those obtained during presence of agonist. The μ-opioid-induced conductance was estimated as a function of membrane potential by dividing the DAMGO-activated current by the difference between the membrane potential and the reversal potential.

INTERNAL SOLUTIONS. The standard pipette internal solution consisted of (in mM) 130 K-gluconate, 5 NaCl, 1 CaCl₂, 1 MgCl₂, 11 ethylene glycol-bis(β-aminoethy]ether)-N,N,N’,N’-tetraace- tic acid (EGTA), 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonylic acid (HEPES), 2 ATP (magnesium salt, equine, Sigma Chemical, St. Louis, MO), 0.1 GTP (lithium salt, Sigma, St. Louis, MO). Other internal solutions were prepared, from which either ATP, GTP, or both were omitted (see Table 1). In some experiments, guanosine-5’-O-(3-thiophosphosphate) (GTP-γ-S; 100 μM; Boehringer-Mannheim, Indianapolis, IN) replaced GTP or guanosine-5’-O-(2-thiodiphosphate) (GDP-β-S; 500 μM; Boehringer-Mannheim) was included in the standard solution. The pH and osmolarity of internal solutions were adjusted to 7.3 and 280–285 mosmol, respectively. Solutions were prepared in advance, stored in 1.5- to 5-ml aliquots at −40 to −80°C and thawed before use on the day of experiment. In some experiments, pipettes were backfilled with standard internal solution containing 0.2–2% bicytin (free base; Sigma, St. Louis, MO) or 0.5% Neurobiotin (Vector Laboratories, Burlingame, CA) to label neurons during recording.

DRUG SOLUTIONS. DAMGO (acetate salt), (+)-α-aminoo-3-hy- droxy-5-methylisoxazole-4-propionic acid (AMPA), naloxone, and tetrodotoxin (TTX) were obtained from Sigma Chemical (St. Louis, MO). DAMGO was dissolved in distilled water and frozen in 1-mg/ml aliquots at −40 to −80°C. Before use, aliquots were thawed and added to a volume of ACSF to obtain the desired final drug concentration. Cesium chloride (2 mM; Sigma Chemical) was added to the perfusate in some experiments.

Histochemical processing

Tissue slices containing biocytin- or neurobiotin-filled neurons were fixed overnight in cold (4°C) phosphate-buffered 4% paraformaldehyde/4% sucrose (pH 7.4) and washed for 12–48 h in 30% sucrose in 0.1 M phosphate buffer (pH 7.35) at 4°C. Slices were frozen-sectioned parallel to the slice surface at 40 μm, pre-treated with ethanol (Metz et al. 1989), reacted with ABC complex
two other cells could not be morphologically characterized because of incomplete staining. Labeled dendrites arborized dorsoventrally within the SG, displaying limited encroachment into adjacent laminae. Dendrites were often observed at the cut tissue surfaces, suggesting that some branches were pruned during slicing. Two stalked cells featured axons that coursed ventrally and gave off bouton-bearing branches in laminae III-V; another cell exhibited a delicate axon-like process that ramified in lamina II. The seventh labeled neuron had stalked cell features but was situated in the inner region of the SG (lamina II) and gave rise to an axon that passed ventromedially into lamina III.

**μ-Opioid actions on hamster and rat SG neurons**

Bath application of DAMGO (1–5 μM) hyperpolarized $V_m$ ($-11.8 \pm 6.4$ mV, $n = 27$) and decreased $R_N$ ($-41.7 \pm 22.8\%$, $n = 27$) in 60% (27/45) of hamster SG neurons recorded with pipettes filled with the standard internal solution containing 2 mM ATP and 100 μM GTP. When applied at concentrations up to 5 μM, DAMGO had no effect on the 14 remaining rat SG neurons.

Under voltage-clamp conditions, application of DAMGO (1 μM) activated a voltage-dependent membrane current $I_{op}$ (10/25 hamster SG cells), revealed by subtracting the current obtained in ACSF from that obtained in the presence of agonist at a given voltage (Fig. 2D). At an extracellular potassium concentration of 2.5 mM, the DAMGO-activated current was outward at membrane potentials more positive than $-80$ mV and reversed to a net inward current at potentials negative to $-80$ mV (Fig. 2E), somewhat more positive than the $V_{rev}$ of $-100$ mV predicted by the Nernst equation. At an elevated extracellular potassium concentration (8.5 mM), this reversal potential was shifted to the right (i.e., more positive; Fig. 2E). The $μ$-opioid-induced conductance was $-5$ nS at $-55$ mV and increased slightly with membrane hyperpolarization to 7 nS at $-115$ mV, which approximates those properties reported for rat locus coeruleus neurons (Williams et al. 1988).

In agreement with previous sharp electrode experiments (Grudt and Williams 1993, 1994), the DAMGO-activated conductance was voltage sensitive, increasing at $V_m$ negative to $-55$ mV (Fig. 2, D and E). Under voltage-clamp conditions and in the absence of DAMGO, a steady-state, inwardly rectifying current was also observed when neurons were stepped to potentials more negative than the resting membrane potential. This current was increased at $V_m$ negative to $-55$ mV by elevating extracellular potassium; conversely, adding cesium (2 mM) to the bathing solution blocked this current (data not shown).

The DAMGO-induced hyperpolarization of hamster
FIG. 2. Effects of bath-applied DAMGO on SG neurons recorded with patch-pipette electrodes. Pipette internal solution contained 2 mM ATP and 100 μM GTP. A: response of an SG neuron to DAMGO (1 μM) applied in normal artificial cerebrospinal fluid (ACSF) and during bath application of 1 μM tetrodotoxin (TTX) to block Na+-dependent synaptic transmission. Superfusate contained drug during periods indicated by filled bars. (V_m, −54 mV). B: response of another SG neuron to DAMGO (0.5 μM) in ACSF and in the presence of 50 nM naloxone. (V_m, −57 mV). Downward deflections in baseline represent passive membrane voltage responses to pulses of current injected through the recording pipette used to measure neuronal input resistance. Calibration in A applies to B. C: DAMGO-activated membrane hyperpolarization of hamster and rat SG cells is dose dependent. Number of experiments used to determine data points in dose-response curves are shown in parentheses. Drug was added to the superfusate for 30-s or 1- to 2-min periods (see text). D and E: steady state current-voltage plots of SG neurons from hamster constructed under voltage clamp were generated by holding cells at −55 mV and stepping the membrane potential to indicated values for 1 s. D: DAMGO (1 μM)-activated membrane current for 5 SG neurons (filled symbols, dashed lines). Current-voltage plot indicated by open circles shows data pooled from all experiments. E: DAMGO-activated membrane current for an SG neuron plotted at 3 different external potassium concentrations (in mM). F: effect of 1 μM DAMGO on spontaneous synaptic currents (SCs) recorded from an SG neuron under voltage clamp. Representative chart recordings before (ACSF) and during (DAMGO) bath application of drug showing inhibition of SCs (downward deflections in traces) by μ-opioid receptor activation. Holding potential, −64 mV.

SG cells was dose dependent, increasing from a mean of −3.0 ± 1.0 mV at a bath concentration of 250 nM to −9.0 ± 1.4 mV at 5 μM (Fig. 2C). Dose-response curves were constructed for hamster SG recordings in which neurons were exclusively presented with 30-s applications of DAMGO. For comparison, curves from rat neurons were based on presentations of DAMGO lasting either 30 s (n = 4) or 1–2 min (n = 5; Fig. 2C). Estimating from these curves, we determined that the ED_{50} for DAMGO in hamster (30-s presentation) was ~0.5 μM, compared with those for rat, which were 0.4 and 0.05 μM for 30 s and 1–2 min presentations, respectively (the latter which we determined was to equilibrium).

In addition to its direct actions on recorded neurons, DAMGO (1–2 μM) also affected the spontaneous inward synaptic currents [presumably excitatory postsynaptic currents (EPSCs)] exhibited by some SG cells. At a holding potential of −60 mV, applying DAMGO to such neurons depressed spontaneous synaptic currents and had no effect on resting membrane current (Fig. 2F). This observation is consistent with the reports of Glaum et al. (1994) and Hori et al. (1992), which noted μ-opioid-mediated inhibition of excitatory transmission in rat superficial dorsal horn.

ATP and GTP appear to be necessary for μ-opioid-activated postsynaptic effects in SG neurons.

By using patch pipettes filled with different internal solutions, we determined that the DAMGO-activated membrane hyperpolarization was sensitive to dialysis of essential stores of ATP and GTP (see Table 1). Several experiments led us to this conclusion.

When we recorded with patch pipettes with internal solution lacking both ATP and GTP (Basic), we observed a DAMGO-induced membrane hyperpolarization in about the same proportion of neurons as we noted in previous experi-
FIG. 3. Effects of altering internal solution composition on μ-opioid activated postsynaptic responses of hamster SG neurons. A and B: chart records show membrane potential (V_m; top trace) and membrane current (I_m; bottom trace). Downward deflections in baselines represent pulses of current injected (bottom) and resulting passive membrane voltage response (top) to measure neuronal input resistance (R_N). Calibration in A applies to B. A: DAMGO (5 μM) hyperpolarized V_m and decreased R_N in SG neuron recorded with standard internal solution (2 mM ATP and 100 μM GTP; see METHODS). At peak DAMGO response, V_m was briefly returned to its original level to compare R_N (V_m, –51 mV). B: DAMGO (5 μM)-activated response of an SG neuron recorded with internal solution without ATP and GTP (V_m, –54 mV). C: effects of DAMGO (1 μM) on an SG neuron recorded with internal solution containing the G-protein inhibitor GDP-β-S. Pipette tip was filled with internal solution containing 2 mM ATP and then backfilled with internal solution containing 500 μM GDP-β-S. Response to 2nd DAMGO application was attenuated by 64% and was nearly absent after a 3rd application. (Horizontal bars below trace indicate magnitude of initial DAMGO response.) D and E: effects of bath-applied DAMGO on 2 SG neurons recorded with electrodes containing basic internal solution with added 2 mM ATP and 100 μM GTP-γ-S, a G-protein activator. Downward deflections in baseline are passive voltage responses to current injected episodically to measure R_N. D: recording pipette tip was filled with GTP-γ-S solution. Initial application of DAMGO (0.5 μM) activated a persistent hyperpolarization of membrane potential (V_m) and decrease in R_N. Asterisk (*) indicates where membrane potential (V_m) was briefly returned to its original level to compare R_N. Subsequent applications of DAMGO and naloxone (1 μM) had no effect on V_m or R_N, consistent with an intracellular locus for GTP-γ-S actions rather than persistent μ-opioid receptor occupancy (initial V_m, –54 mV). E: recording pipette tip filled with standard ATP- and GTP-containing solution and pipette backfilled with solution containing 100 μM GTP-γ-S. Initial application of DAMGO (0.5 μM) activated a reversible membrane hyperpolarization. Second DAMGO application was followed by prolonged hyperpolarizing response and decrease in R_N (arrow) as GTP-γ-S diffused into the neuron (initial V_m, –52 mV). F: summary of μ-opioid-activated postsynaptic responses of hamster SG neurons as a function of internal pipette solution constituents. Internal solutions indicated below each histogram contained both ATP and GTP, GTP, or omitted both nucleotide triphosphates (BASIC). GDP-β-S (500 μM) was added to the ATP- and GTP-containing solution (GDP/βS). (See METHODS for details.) Left: percentage of neurons responding to DAMGO (1–5 μM). Number of neurons recorded under the various conditions is given in parentheses. Middle: maximum membrane hyperpolarization reached during DAMGO responses. Right: associated DAMGO-activated decrease in neuronal input resistance (R_N) relative to control. * Different from control (ATP/GTP), at P < 0.05 (t-test).
Fig. 4. μ-Opioid responsiveness of hamster SG neurons during course of whole cell recording as a function of internal pipette solution (see Methods and Fig. 3F for details). Magnitude of DAMGO-activated (1 μM) hyperpolarization is plotted vs. the time elapsed from initiation of whole cell recording configuration (n = 10 cells). A control membrane response to an initial application of DAMGO was made for each cell ±3 min after acquisition of recording. Effect of subsequent DAMGO applications on membrane potential was tested at 10- to 20-min intervals, expressed relative to the first (control) response and normalized across the population. Responses indicated by asterisk obtained from the intracellularly stained neuron shown in Fig. 1B.

DISCUSSION

Unlike previous whole cell recording studies, but consistent with experiments using sharp micropipette electrodes, this study, employing whole cell recording methodology, demonstrates that the μ-opioid agonist DAMGO activated an inwardly rectifying potassium conductance in 60% of hamster neurons and 39% of rat neurons sampled from the lumbar spinal SG. Our results also indicate that spinal SG neurons exhibit a pronounced sensitivity to dialysis of intracellular GTP and ATP stores by the patch pipette. The ED₅₀ of DAMGO in rats was ~50 nM, well within the physiologic range of systemically applied μ-opioids. This range probably applies to the hamster as well because 30 s applications of DAMGO in the rat experiments tissue led to an ED₅₀ that was similar to hamster neurons (see Fig. 2C).

Ionic basis of μ-opioid-induced voltage changes in hamster SG neurons

Consistent with previous reports, we determined that opioids activate a receptor that induces membrane hyperpolarization in SG neurons via activation of a potassium conductance (Grudt and Williams 1993, 1994; Jeftinija 1988; Murase et al. 1982; Yoshimura and North 1983). A μ-opioid-activated potassium conductance having many of the same properties as that observed in SG neurons has been investigated more thoroughly in locus coeruleus neurons. This potassium conductance is voltage sensitive and inwardly rectifying, becoming larger at V_m more negative than −60 mV (Grudt and Williams 1993, 1994; North and Williams 1985; Williams et al. 1988). In addition to its activation by opioids, this inward rectifying current is enhanced by other ligands, such as somatostatin (Mihara et al. 1987) and norepinephrine (Miyake et al. 1989; Shen et al. 1992; Surprenant and North 1988). However, the mechanism coupling opioid-receptor activation to this potassium channel is only partially shared by these other ligands (Reisine et al. 1996). Others found opioid coupling to a similar potassium channel in hippocampus but also found evidence of coupling to a voltage-gated potassium channel of a very different type (Wimpey and Chavkin 1991). We did not observe evidence of a channel of this latter type in the current study.

Opioid sensitivity of SG neurons

Studies employing sharp microelectrode recordings to examine opioid actions on similar spinal cell populations in rats reported varying proportions of opioid-sensitive cells. Although we found that 60% of sampled SG cells in hamsters and 39% in rats were responsive to DAMGO, Yoshimura and North (1983) reported that 50% of lamina II neurons were hyperpolarized by enkephalin, whereas Jeftinija (1988) later observed that 75% of such neurons were so affected. Grudt and Williams (1994) reported that 86–90% of neurons in the trigeminal SG of guinea pig and rat, respectively, were hyperpolarized by enkephalin. These reported variations in opioid sensitivity may reflect sharp versus patch...
electrode sampling biases, different opioid agonists, regional differences (trigeminal vs. spinal cord), or age-related influences on opioid expression among species.

In cat and monkey, lamina II can be subdivided into distinct outer (IIo) and inner (IIn) cytoarchitectonic regions, which have been shown to receive terminations from afferent fibers signaling predominantly noxious and innocuous inputs, respectively (Kumazawa and Perl 1978; Light et al. 1979). In our experiments, attempts were made to position recording pipettes within the translucent region of the superficial dorsal horn corresponding to lamina II. Intracellular labeling of representative neurons suggested that our recordings sampled mainly from stalked cells, predominantly in a region that is associated with direct synaptic input from nociceptors (Light 1992). It was not possible in our isolated preparation to directly characterize recorded cells on the basis of functional characteristics of their afferent input. We therefore could not determine if there was any correlation between μ-opioid responsiveness and activation by noxious inputs.

Intracellular staining also indicated that the dendritic trees, which are generally elongated rostrally and caudally in other species (Beal and Cooper 1978; Light et al. 1979; Scheibel and Scheibel 1968), could have been truncated by the transverse plane of sectioning. However, because we found no correlation between DAMGO responsiveness and distance of recorded neurons from the slice surface, it is unlikely that differences in opioid sensitivity reported here are due to pruning of dendritic μ-opioid receptors. We conclude that the cell-to-cell variation in DAMGO responsiveness reported here is a function of μ-opioid receptor expression by lamina II neurons and is not an artifact resulting from preparation of isolated tissue.

Our sample of SG neurons in hamsters and rats, recorded in whole cell configuration, had an average input resistance three- to fourfold higher than values reported previously in sharp microelectrode recordings from mixed populations of dorsal horn neurons in hamsters (Schneider and Perl 1988) and lamina II neurons in rat (Baba et al. 1994; Murase and Randic 1983; Yoshimura and Jessell 1989; Yoshimura and North 1983). The difference in Rm reported here and by others is consistent with a comparison between whole cell and microelectrode recordings of hippocampal dentate gyrus granule cells (Staley et al. 1992), indicating that the whole cell recording configuration produces less current shunt because of higher seal resistance between pipette and neuronal membrane. Assuming a somatic recording location and that opioid receptors are distributed on the somatic and proximal dendritic membrane of SG neurons, a smaller shunt pathway would be expected to facilitate detection of small membrane potential changes induced by μ-opioids. More likely, the proportion of opioid-sensitive cells reported here is influenced by dilution of unknown diffusible factors required for opioid receptor-response coupling via exchange between intracellular milieu and internal pipette solution.

Previous whole cell recording studies failed to report postsynaptic effects of applying opioids to SG neurons (Hori et al. 1992), presumably because the μ-opioid effects are reduced in neurons if ATP and GTP are omitted from the pipette internal solution (e.g., Forscher and Oxford 1985). Glaum et al. (1994) included ATP (but no GTP) in patch pipettes and observed a DAMGO-evoked outward current. However, it was reported that few rat SG neurons exhibited this response, that it required relatively high concentrations of DAMGO, and that the current quickly desensitized with repeated agonist applications. Our results in hamsters and rats suggest that intracellular stores of GTP associated with possible activation of G-coupled proteins are depleted from SG neurons unless replenished during whole cell recording by inclusion within the pipette internal solution.

It is not known whether μ-opioid effects observed in other slice preparations are also dependent on intracellular ATP and/or GTP (e.g., Grudt and Williams 1994; Williams et al. 1988) because such studies commonly employed sharp microelectrodes, which would not be expected to alter the intracellular environment to the same degree as whole cell recordings. Williams et al. (1988), however, reported that μ-opioid-activated outward currents in rat locus coeruleus neurons were reduced by intracellular injection of GTP-γ-S, but not GDP-β-S, through the recording micropipette. This observation and other evidence (Miyake et al. 1989) suggest that coupling between μ-opioid receptors and membrane potassium channels in locus coeruleus neurons may not be as sensitive to alterations in levels of intracellular mediators as the current results would suggest about μ-receptor-channel coupling in hamster SG neurons.

**Postsynaptic versus presynaptic effects of μ-opioids**

Previous studies as well as this study demonstrated that opioids decrease the frequency of EPSCs recorded in SG neurons at physiologically relevant doses (Glaum et al. 1994). Our results indicate that DAMGO activates a direct membrane hyperpolarization in rat SG neurons with an ED50 of ~0.05 μM. The ED50 reported for DAMGO-produced inhibition of EPSCs in rat SG was 0.5 μM (Glaum et al. 1994), indicating that direct actions of opioids on postsynaptic membrane are at least as potent as presynaptic effects. The relative contribution of these direct opioid effects on analgesia is further complicated by reported indirect effects; μ-opioids reduce or enhance NMDA receptor-mediated activation of SG neurons by binding to NMDA receptors in a nonopioid-receptor-mediated manner (Chen and Huang 1991; Chen et al. 1995a,b); μ-opioids in dissociated SG neurons also reduce AMPA-activated currents in an opioid selective fashion (Kolaj and Randic 1996); and μ-opioids have been shown to enhance GABA_A-receptor-mediated currents in isolated dorsal horn neurons (Wang and Randic 1994).

**Functional significance**

These data are consistent with other studies reporting that physiologically significant doses of μ-opioids activate a postsynaptic, potassium-dependent conductance in a subset of spinal lamina II neurons. This conductance change causes a hyperpolarization of resting Vm, driving the neuronal membrane away from threshold for action potential initiation, and also shunts excitatory currents away from the initial segment, both of which render the neuron less responsive to excitatory afferent input.

Moreover, μ-opioids appear to exert presynaptic effects,
OPIOID EFFECTS ON SPINAL NEURONS

which can be observed in the same neurons, inhibiting the spontaneous excitatory currents, likely reflecting actions on primary afferent neurons. It appears that both pre- and postsynaptic actions can combine to produce a powerful inhibition of excitation in many laminae I and II neurons. Because many laminae I and II neurons are important in nociceptive processing, this inhibition may mediate the relatively selective analgesia produced by spinal or systemic application of μ-opioids.

We did not observe μ-opioid-induced excitation of any SG neurons in the hamster or rat spinal slice preparation. This is somewhat surprising, given reports that inhibitory interneurons in many regions can be inhibited by μ-opioids, leading to disinhibition of cells on which such neurons terminate (Johnson and North 1992; Magnuson and Dickenson 1991; Neumaier et al. 1988). μ-Opioids were reported to exert excitatory effects on some rat laminae I and II neurons (Jones et al. 1990; Magnuson and Dickenson 1991) and to increase release of substance P from primary afferent neurons (Suarez-Roca and Maixner 1992, 1993, 1995; Suarez-Roca et al. 1992). Although we have no evidence of direct excitatory effects of DAMGO on SG neurons, we did not examine μ-opioid effects on synaptic activation of cells in our slice preparation.

Together the data presented here indicate that a large percentage of SG neurons in the hamster and rat are significantly hyperpolarized by physiologically relevant doses of the opiate receptor agonist DAMGO, making it likely that at least part of μ-opioid analgesic effects are due to a postsynaptic mechanism. These data also demonstrate the sensitivity of the opioid response of SG neurons to the dilution of intracellular stores of GTP. This important finding may explain why previous studies failed to observe these effects.

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REFERENCES
Agahajanian, G. K. and Rasmussen, K. Intracellular studies in the facial nucleus illustrating a simple new method for obtaining viable motoneu-
rons in adult rat brain tissue. H. Willcockson and B. Taylor-Blake provided assistance with preparation of the manuscript.


Blanton, M. G., Lo Turco, J. J., and Kriegstein, A. R. Whole cell recording from neurons in slices of reptilian and mammalian cerebral cor-


Chen, G. G., Chalazonitis, A., Shen, K. F., and Crain, S. M. Inhibitor of cyclic AMP-dependent protein kinase blocks opioid-induced prolonga-
tion of the action potential of mouse sensory ganglion neurons in dissoci-


Duggan, A. W. and North, R. A. Electrophysiology of opioids. Pharma-

Forscher, P. and Oxford, G. S. Modulation of calcium channels by nor-

Glaum, S. R., Miller, R. J., and Hammond, D. L. Inhibitory actions of δ-

Grudt, T. J. and Williams, J. T. δ-Opioid receptors also increase potas-

Grudt, T. J. and Williams, J. T. μ-Opioid agonists inhibit spinal trige-


Kolai, M., and Randic, M. μ-Opioid receptor-mediated reduction of α-


Light, A. R., Traving, D. L., and Perl, E. R. Morphological features of functionally defined neurons in the marginal zone and substantia gal-


Moises, H. C., Rusin, K. I., and MacDonald, R. L. μ-Opioid receptor-

Murase, K., Nedeljkov, V., and Randic, M. The actions of neuropeptides Downloaded from http://jn.physiology.org/ by 10.220.32.246 on October 9, 2016 on October 9, 2016.


