Morphine-3β-d-glucuronide Suppresses Inhibitory Synaptic Transmission in Rat Substantia Gelatinosa

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ABSTRACT

High doses of intrathecally applied morphine or morphine-3β-d-glucuronide (M3G) produce allodynia and hyperalgesia. Whole-cell patch-clamp recordings were made from substantia gelatinosa neurons in transverse slices of adult rat lumbar spinal cord to compare the actions of M3G with those of the μ-opioid agonist, DAMGO ([D-Ala²,N-Met-Phe⁴,Gly-ol⁵]-enkephalin), and the ORL₁ agonist, nociceptin/orphanin FQ (N/OFQ). M3G (1–100 μM) had little or no effect on evoked excitatory postsynaptic currents (EPSC) and no effect on postsynaptic membrane conductance. In contrast, 1 μM DAMGO and 1 μM N/OFQ reduced the amplitude of evoked EPSCs and activated an inwardly rectifying K⁺ conductance.

M3G did not attenuate the effect of DAMGO or N/OFQ on evoked EPSC amplitude. However, 1 to 100 μM M3G reduced the amplitude of evoked GABAergic and glycineergic inhibitory postsynaptic current (IPSC) by up to 48%. This effect was naloxone-insensitive. The evoked IPSC was also attenuated by DAMGO, but not by N/OFQ. Because M3G reduced the frequency of tetrodotoxin-insensitive miniature IPSCs and increased paired-pulse facilitation, it appeared to act presynaptically to disinhibit substantia gelatinosa neurons. This effect, which does not appear to involve μ-opioid or ORL₁ receptors, may contribute to the allodynia and hyperalgesia observed after intrathecal application of high doses of morphine.

Morphine is widely used for the management of moderate to severe pain. It is converted by glucuronidation into two major metabolites, morphine-3β-d-glucuronide (M3G) and morphine-6β-d-glucuronide (M6G) (Boerner et al., 1975; Christrup, 1997). M6G has high affinity for the μ-opioid receptor (Pasternak et al., 1987; Paul et al., 1989; Löser et al., 1996) and appears to be a more potent opioid agonist than morphine (Pasternak et al., 1987; Paul et al., 1989; Frances et al., 1992; Osborne et al., 2000). In contrast, M3G does not bind to μ, δ, or κ-opioid receptors (Pasternak et al., 1987; Lambert et al., 1993; Löser et al., 1996) and appears to be devoid of analgesic activity (Pasternak et al., 1987; Yaksh and Harty, 1988). Furthermore, M3G does not interact with N-methyl-d-aspartate, GABA₂, or glycine receptors (Bartlett et al., 1994) and has no effect on membrane conductance or action potential discharge in locus coeruleus neurons (Osborne et al., 2000). M3G also does not affect Aβ- or C-fiber-evoked responses in dorsal horn neurons (Sullivan et al., 1989; Hewett et al., 1993). It does however produce hyperalgesia and allodynia when administered intrathecally or intracerebroventriculantly (Woolf, 1981; Yaksh et al., 1986; Yaksh and Harty, 1988) and progressively higher doses can cause seizures (Smith et al., 1990; Halliday et al., 1999). These findings are consistent with the suggestion that morphine metabolites may be responsible for the development of hyperalgesia, allodynia, and myoclonus during clinical opioid therapy (De Conno et al., 1991; Sjogren et al., 1998).

Therefore, the aim of the present study was to examine the cellular effects of M3G on neurons in the rat substantia gelatinosa. Actions of M3G were compared with those of the μ-opioid agonist, DAMGO ([D-Ala²,N-Met-Phe⁴,Gly-ol⁵]-enkephalin), and the ORL₁ agonist, nociceptin/orphanin FQ (N/OFQ). Although it is established that M3G does not interact with μ, δ, or κ-receptors, we sought to examine possible interactions with other mechanisms within the dorsal horn, including the more recently defined ORL₁ receptor (Meunier et al., 1995; Reinscheid et al., 1995). Some of these findings have been communicated to the Society for Neuroscience (Moran and Smith, 2000).

ABBREVIATIONS: M3G, morphine-3β-D-glucuronide; M6G, morphine-6β-D-glucuronide; GABA, γ-aminobutyric acid; AP5, DL-2-amino-5-phosphonovaleric acid; CNQX, 6-cyano-7-nitroquinolinoxide-2,3-dione; DAMGO, [D-Ala²,N-Met-Phe⁴,Gly-ol⁵]-enkephalin; EPSC, excitatory postsynaptic current; IPSC, inhibitory postsynaptic current; mIPSC, miniature IPSC; N/OFQ, nociceptin/orphanin FQ; TTX, tetrodotoxin; HPLC, high-performance liquid chromatography; CSF, cerebrospinal fluid; QX-314, N-(2,6-dimethylphenyl)acetamide-2-triethylammonium bromide.
Materials and Methods

Spinal Cord Slice Preparation. All procedures were carried out in compliance with the guidelines of the Canadian Council for Animal Care, the University of Alberta Health Sciences Laboratory Animal Services Welfare Committee, and the Committee for Research and Ethical Issues of the International Association for the Study of Pain.

Sprague-Dawley rats (14–35 days old) were deeply anesthetized with urethane (1.5 g/kg, i.p.). A laminectomy was performed, and ~2 cm of spinal cord with attached ventral and dorsal rootlets was transferred into ice-cold oxygenated (95% O2:5% CO2) dissection solution containing 118 mM NaCl, 2.5 mM KCl, 26 mM NaHCO3, 1.3 mM MgSO4, 1.2 mM NaH2PO4, 1.5 mM CaCl2, 5 mM MgCl2, 25 mM D-glucose, and 1 mM kynurenate. The dura matter was removed, and the spinal cord was glued to an agar block with cyanoacrylate glue. Transverse slices (300 μm) were cut using a Vibratome (Pelco International, Reading, CA) in ice-cold dissection solution and were then incubated at room temperature (22–24°C) in oxygenated dissection solution (see above, without 1 mM kynurenate acid) for 1 h before recording.

Recording and Stimulation. Spinal cord slices were superfused (flow rate ~2–4 ml/min) at room temperature (22–24°C) with 95% O2:5% CO2 saturated artificial CSF (127 mM NaCl, 2.5 mM KCl, 1.2 mM NaH2PO4, 26 mM NaHCO3, 1.3 mM MgSO4, 2.5 mM CaCl2, and 25 mM D-glucose, pH 7.4). For recording excitatory postsynaptic currents (EPSCs), 10 μM bicuculline and 1 μM strychnine were included to block inhibitory synaptic inputs. For recording inhibitory postsynaptic currents (IPSCs), 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 50 μM 6,7-diamo-5-phosphonovaleric acid (AP5) were included to block excitatory synaptic inputs. The GABAergic component of the IPSC was observed in the presence of CNQX and AP5, plus strychnine, whereas the glycinergic component was observed in the presence of CNQX and AP5, plus bicuculline. Tetrodotoxin (TTX; 1 μM) was included when recording miniature IPSCs (mIPSC).

The substantia gelatiosa was identifiable as a translucent band across the dorsal horn. Whole-cell recordings were made with an NPI SEC 05L amplifier (NPI Electronic GmbH, Tamm, Germany) in discontinuous single-electrode voltage-clamp or bridge-balance current-clamp mode using either the “blind” whole-cell patch-clamp technique or from visually identified substantia gelatinosa neurons using infrared-differential interference contrast video microscopy. Patch pipettes were pulled from thin-walled borosilicate glass (WPI, Sarasota, FL). These had resistances of 5 to 10 MΩ when filled with an internal solution containing 130 mM potassium gluconate, 1 mM MgCl2, 2 mM CaCl2, 10 mM HEPES, 10 mM EGTA, 4 mM Mg-ATP, and 290–300 mM Na-GTP, pH 7.2. In some experiments, the sodium channel blocker QX-314 (5 mM) was included in the internal solution to prevent action potential discharge. Resting membrane potential was typically between ~55 to ~65 mV in bridge-balance current-clamp mode. For voltage-clamp experiments, membrane potential was clamped at ~60 or ~70 mV for recording EPSCs and 0 mV for recording IPSCs. Switching frequencies were typically between 30 and 40 kHz. Signals were filtered at 2 kHz and digitized between 5 and 10 kHz. EPSCs were evoked at 0.05 Hz with a bipolar concentric stimulating electrode (FHC Inc., Bowoinham, ME) that was placed on the dorsal root or near the dorsal root entry zone to activate primary afferent fibers. Stimulus duration was 100 or 400 μs. IPSCs were evoked at 0.05 Hz by focal stimulation with a patch pipette containing artificial CSF. For paired-pulse experiments, IPSCs were evoked at 0.05 Hz with two paired stimuli (interstimulus interval 50–100 ms), and 10 consecutive responses were averaged for analysis. EPSCs and IPSCs were identified as being monosynaptic by their ability to follow high frequency stimulation (10 or 20 Hz) with constant latency and the absence of failures. An Axopatch 1D (Axon Instruments, Inc., Foster City, CA) was used for recording mIPSCs, and data were only used if the series resistance was below 25 MΩ. Currents were filtered at 1 kHz and digitized at 5 kHz, and data were stored on disk.

Data Analysis. Data were acquired and analyzed using pCLAMP 8.0 (Axon Instruments, Inc.). Statistical comparisons were made using GraphPad Instat 3.05 (GraphPad Software Inc., San Diego, CA). mIPSCs were analyzed using Mini Analysis Program (Synapsoft, Decatur, GA) on the basis of amplitudes exceeding a threshold set above the baseline noise of the recording. Between 500 and 1000 individual mIPSC were analyzed for each cell. Detected events were reexamined visually and either accepted or rejected. The program was used to measure amplitudes and interevent intervals (frequency) and cumulative probability plots were constructed. Statistical analysis for each neuron was performed using the Kolmogorov-Smirnov nonparametric test. Distributions were considered statistically different if p < 0.05. Figures were produced with Origin 6.1 (OriginLab Corp., Northampton, MA) or Igor Pro 3.1 (WaveMetrics, Lake Oswego, OR).

Drugs and Chemicals. Drugs were applied by bath superfusion for 5 to 6 min. This was long enough for equilibration with the tissue, as drug responses to 5- to 6-min applications were no longer with longer application times (up to 20 min). DAMGO, naloxone, and strychnine were obtained from Sigma-Aldrich (St. Louis, MO). Nociceptin, bicuculline, CNQX, and AP5 were from Tocris Cookson Inc. (Bailwin, MO). QX-314 was supplied by AstraZeneca Pharmaceuticals LP (Wilmington, DE) and TTX was from Alomone Labs (Jerusalem, Israel). Morphine sulfate was from British Drug Houses (Toronto, ON, Canada). M3G was from Lipomed (Arlesheim, Switzerland) and contained 0.28% morphine (HPLC analysis, Neurochemistry Research Unit, University of Alberta, Canada).

Results

Morphine-3β-D-glucuronide Does Not Affect Evoked Excitatory Postsynaptic Currents. Whole-cell patch-clamp recordings were obtained from substantia gelatinosa neurons from slices maintained in vitro for up to 10 h and stable recordings were made from individual neurons for up to 3 h.

In the presence of 10 μM bicuculline and 1 μM strychnine, stimulation of the dorsal root or dorsal root entry zone generated EPSCs in substantia gelatinosa neurons at a holding potential of ~60 or ~70 mV. Superfusion of 1 to 100 μM M3G had no significant effect on EPSC amplitude in any of the 19 cells tested. By contrast, and confirming previous reports (Glaum et al., 1994; Kohno et al., 1999), the μ-opioid agonist DAMGO (1 μM) reduced EPSC amplitude by an average of 46.9 ± 4.82% (n = 19/25 cells tested). Similarly, the ORL1 agonist N/OFQ (1 μM) reduced EPSC amplitude by 39.6 ± 7.10% (n = 10/5/6 cells tested), which confirms the findings of Liebel et al. (1997) and Lai et al. (1997). Sample data records are shown in Fig. 1. In Fig. 1A the EPSC is unaffected by M3G but is suppressed by N/OFQ. In Fig. 1B, M3G is again ineffective but the EPSC is suppressed by DAMGO. Time courses of the effects of these drugs are shown in Fig. 1, C and D. The histogram in Fig. 1E summarizes the effects of M3G, DAMGO, and N/OFQ on evoked EPSCs.

Morphine-3β-D-glucuronide Does Not Interact with ORL1, or μ-Opioid Receptors in Substantia Gelatinosa Neurons. Because M3G had no noticeable effect on excitatory transmission at synapses where N/OFQ and DAMGO suppressed transmission, it is unlikely to act as an ORL1 or μ-opioid agonist. M3G was also devoid of antagonist activity at these receptors. Superfusion of 1 or 10 μM M3G did not occlude the effects of N/OFQ. In the presence of M3G, superfusion of 1 μM N/OFQ reduced EPSC amplitude by 41.8 ±
7.84% \( (n = 3; \text{Fig. 2A}) \), which is similar to the actions of \( \text{N/OFQ} \) by itself on EPSC amplitude \( (p > 0.85, t \text{ test} ; \text{compare with Fig. 1A}) \). In a similar series of experiments, superfusion of \( 1 \mu \text{M M3G} \) did not occlude the actions of \( \text{DAMGO} \). In the presence of \( \text{M3G} \), superfusion of \( \text{DAMGO} \) reduced EPSC amplitude by 40.9 ± 4.30% \( (n = 3; \text{Fig. 2B}) \), which is similar to the actions of \( \text{DAMGO} \) alone \( (p > 0.85, t \text{ test} ; \text{compare with Fig. 1B}) \). Figure 2, C and D, shows time courses of these drug effects on EPSC amplitude. These observations are consistent with binding studies that show that \( \text{M3G} \) does not bind to \( \mu \)-opioid receptors (Pasternak et al., 1987; Lambert et al., 1993; Löser et al., 1996).

**Comparison of Postsynaptic Actions of Morphine-3β-d-glucuronide, Nociceptin/Orphanin FQ, and DAMGO.**

Membrane conductance measured from a voltage-ramp protocol was unaffected by \( \text{M3G} \) (1 \( \mu \text{M}, n = 11 \text{ or } 100 \mu \text{M}, n = 5 \). Figure 3A shows the lack of effect of 100 \( \mu \text{M M3G} \) on currents evoked by a voltage ramp from −140 to 0 mV. In the same cell (Fig. 3B), 1 \( \mu \text{M DAMGO} \) increased conductance at negative voltages, reflecting its activation of an inwardly rectifying conductance (Grudt and Williams, 1994; Schneider et al., 1998). \( \text{M3G} \) (1 \( \mu \text{M} \) also had no effect on excitability \( (n = 5) \), as evaluated by the frequency of action potential discharge in response to depolarizing current pulses (data not shown). These findings are similar to those of Osborne et al. (2000) who found no effect of \( \text{M3G} \) on membrane conductance or action potential firing in locus coeruleus neurons.

In contrast to the lack of effect of \( \text{M3G} \) on membrane conductance, \( \text{N/OFQ} \) (1 \( \mu \text{M}, 8/14 \text{ cells} \) activated an inwardly rectifying conductance, which was reflected by a 49.0 ± 5.98 pA increase in current at −140 mV (Fig. 3C). This was very similar to the findings in medullary dorsal horn (Jennings, 2001). The reversal potential for the \( \text{N/OFQ} \)-induced current of −97.0 ± 2.51 mV in 2.5 mM \( [\text{K}^+]_o \), \( n = 8 \) was shifted to −75.9 ± 4.28 mV in 6.5 mM \( [\text{K}^+]_o \), \( n = 3 \), consistent with the activation of a \( \text{K}^+ \) conductance. \( \text{DAMGO} \) (1 \( \mu \text{M} \) also acti-
vated an inwardly rectifying K⁺ current of 60.5 ± 10.0 pA (n = 5/12 cells tested) at −140 mV (Fig. 3D), which confirms previous reports (Grudt and Williams, 1994; Schneider et al., 1998).

Actions of Morphine-3β-D-glucuronide on Evoked and Miniature Inhibitory Postsynaptic Currents. In the presence of the glutamate receptor antagonists, 50 μM AP5 and 10 μM CNQX, focal stimulation generated IPSCs in substantia gelatinosa neurons at a holding potential of 0 mV. In contrast to its lack of effect on evoked EPSCs, M3G produced a concentration-dependent decrease in the amplitude of the evoked IPSC (Fig. 4A). Sample data records are illustrated in Fig. 4B. Because HPLC analysis indicated that our M3G contained ~0.28% morphine, it was possible that the effect on the IPSC was caused by the small amount of morphine in our sample. Therefore, the effect of 100 μM M3G was compared with that of 300 nM morphine. This low concentration of morphine caused a negligible reduction in IPSC amplitude (9.73 ± 6.19%, n = 3; Fig. 4, B and C). Figure 4C shows the time course of the effect of 100 μM M3G on IPSC amplitude. It was also possible that our sample of M3G was contaminated with a small amount of M6G, which is a potent μ-agonist (Osborne et al., 2000). To test for this possibility, effects of 100 μM M3G on the IPSC were studied in the presence of 100 μM naltrexone. Data records are shown in Fig. 4D and the time course of the effect of M3G in the presence of naloxone is shown in Fig. 4E. Because the effect of M3G was unchanged, the actions of M3G do not reflect contamination of the sample by μ-agonists. Moreover, they confirm that the action of M3G is not mediated via μ- and κ-opioid receptors.

To further characterize the action of M3G on inhibitory synaptic transmission, we examined the effect of 100 μM M3G on TTX-insensitive mIPSCs. M3G (100 μM) reduced the frequency (n = 4/4 cells tested, Kolmogorov-Smirnov test, p < 0.05; Fig. 5, A–C), but had no effect on the amplitude of the mIPSCs (n = 4/4 cells tested; p > 0.05, Kolmogorov-Smirnov test; Fig. 5C). This preferential effect on mIPSC frequency rather than amplitude suggested that M3G acted presynaptically. Additional evidence for a presynaptic site of action of M3G was obtained from paired-pulse experiments. Two identical stimuli separated by an interstimulus interval (50–100 ms) resulted in paired-pulse facilitation of the evoked IPSC.

Fig. 2. M3G is not an antagonist at ORL₁ or μ-opioid receptors in substantia gelatinosa neurons. EPSCs were evoked by stimulating the dorsal root in the presence of 10 μM bicuculline and 1 μM strychnine. A and B, averaged traces (n = 3) of evoked EPSCs from a holding potential of −60 mV. M3G (10 μM) does not affect N/OFQ or DAMGO-induced suppression of the evoked EPSC. C and D, time course of changes in the amplitude of evoked EPSCs. Graph in C refers to cell illustrated in A, and graph in D refers to that in B.
The mean ratio of the amplitude of the paired IPSCs was $1.04 \pm 0.09$ (IPSC$_2$/IPSC$_1$, $n = 4$). In four of six cells, superfusion of M3G (100 $\mu$M M3G) produced an increase in the mean ratio of IPSC$_2$/IPSC$_1$ to $1.67 \pm 0.45$ (paired $t$ test; $n = 4$). Sample data records are shown in Fig. 6A. In Fig. 6B, the IPSCs have been normalized to the amplitude of IPSC$_1$ to better illustrate the change in the paired-pulse ratio. Figure 6C is a summary histogram of the effect of 100 $\mu$M M3G on the paired-pulse ratio.

Evoked IPSCs in the spinal cord comprise GABAergic and glycinergic components. To examine whether M3G selectively affected one of these components, we examined its effect on evoked GABAergic IPSCs in the presence of AP5, CNQX, and strychnine (1 $\mu$M) and glycinergic IPSCs in the presence of AP5, CNQX, and bicuculline (10 $\mu$M). Both components of the IPSC were similarly affected. Thus, in four of four cells tested, 100 $\mu$M M3G suppressed the GABAergic IPSC by $48.7 \pm 12.8\%$ ($p < 0.05$) and in four of five cells tested, it suppressed the glycinergic IPSC by $39.2 \pm 4.8\%$ ($p < 0.05$). Sample data records for GABAergic and glycinergic IPSCs are shown in Fig. 7, A and B, respectively. Summary histograms are shown in Fig. 7, C and D.
Fig. 4. M3G inhibition of evoked IPSCs in substantia gelatinosa neurons. IPSCs were evoked at a holding potential of 0 mV by focal stimulation in the presence of 50 µM AP5 and 10 µM CNQX. A, a log-concentration response curve illustrating the concentration-dependent action of M3G on the IPSC. B, superimposed, averaged traces (n = 3) of evoked IPSCs before and during application of 100 µM M3G (top panel). Note that M3G strongly inhibits the IPSC. Superimposed, averaged traces (n = 3) of evoked IPSCs before and during application of 300 nM morphine (bottom panel). This low concentration of morphine was without effect. Traces shown in top and bottom panels were obtained from the same neuron. C, time course of changes in the amplitude of evoked IPSCs during application of morphine and M3G. D, superimposed, averaged traces (n = 6) of evoked IPSCs before and during application of 100 µM M3G in the presence of 100 µM naloxone. E, time course of changes in the amplitude of evoked IPSCs during application of M3G and naloxone.
Actions of N/OFQ and DAMGO on Evoked Inhibitory Postsynaptic Currents. Recently, N/OFQ has been reported to selectively suppress glutamatergic synaptic inputs in the spinal cord dorsal horn (Zeilhofer et al., 2000; Ahmadi et al., 2001) but to have no effect on inhibitory synaptic currents. We observed a similar lack of effect of N/OFQ (1 μM) on evoked IPSCs. N/OFQ reduced the amplitude of evoked IPSCs by only 5.88 ± 0.75% (p < 0.05, n = 5/5 cells tested; Fig. 8A). The actions of N/OFQ on synaptic transmission in the substantia gelatinosa are the reverse of M3G, which inhibits IPSCs, but fails to affect EPSCs. Furthermore, 1 μM DAMGO reduced IPSC amplitude (49.6 ± 10.8%, n = 6/10 cells tested, p < 0.05; Fig. 8B) confirming the findings of Grudt and Henderson (1998) but contradicting those of Kohno et al. (1999), who found that DAMGO did not inhibit IPSCs in rat lumbar dorsal horn. Figure 8C shows the time course of the effect of DAMGO on IPSC amplitude. Figure 8D is a summary histogram that compares the effect of DAMGO, M3G, and N/OFQ on IPSC amplitude.

Discussion

In this study, the cellular effects of M3G were compared with those of the ORL₁ agonist N/OFQ and the μ-opioid agonist DAMGO in substantia gelatinosa neurons of rat lumbar spinal cord. M3G had little or no effect on excitatory synaptic transmission at synapses where ORL₁ or μ-opioid agonists were effective. M3G also failed to affect postsynaptic membrane conductance or excitability, whereas, both N/OFQ and DAMGO activated an inwardly rectifying K⁺ conductance. Moreover, suppression of excitatory synaptic responses by N/OFQ or DAMGO was not antagonized by M3G. M3G is therefore neither an agonist nor antagonist at ORL₁ or μ-opioid receptors. However, M3G produced a naloxone-insensitive, concentration-dependent suppression of inhibitory synaptic transmission. The GABAergic and glycinergic components of the IPSC were similarly affected. Analysis of TTX-insensitive mIPSCs indicated that this action of M3G was presynaptic. This finding was supported by paired-pulse experiments where M3G produced an increase in paired-pulse facilitation.

The lack of effect of 1 to 100 μM M3G on EPSCs is consistent with binding studies (Bartlett et al., 1994) and neurochemical assays that showed M3G has no affinity for the N-methyl-D-aspartate receptor and does not affect the release of glutamic acid from synaptosomes (Bartlett and Smith, 1996). Moreover, intrathecal M3G has no effect on C-fiber-
inhibitory transmission was not caused by morphine contamination of our sample of M3G because a concentration of morphine equivalent to the amount of contamination had no effect. Involvement of potential μ-agonist contaminants (morphine and M6G) was also ruled out by the lack of effect of naloxone on M3G-induced suppression of IPSCs.

Because intrathecally administered GABA_A and glycine receptor antagonists (Beyer et al., 1985; Yaksh et al., 1986; Kaneko and Hammond, 1997; Zhang et al., 2001) have pronociceptive actions similar to M3G, selective suppression of inhibitory synaptic transmission by M3G may explain its allodynic and hyperalgesic effects. It may also explain the allodynia, hyperalgesia, and myoclonus observed after administration of high-dose morphine in humans (De Conno et al., 1991; Sjogren et al., 1993, 1994, 1998; Heger et al., 1999). Our observed effects may be especially relevant to palliative care situations, where heroic doses of morphine (up to 20 g/day) are required to produce analgesia in tolerant individuals (Hagen and Swanson, 1997; Sjogren et al., 1998). In humans, intrathecal injection of 1100 mg of morphine results in an accumulation of ~3 μM M3G in CSF (Goucke et al., 1994). By extrapolation, the concentration of M3G in the CSF of a palliative care patient who had received 20 g of morphine within a day would approach 60 μM (Hagen and Swanson, 1997; Sjogren et al., 1998). This falls within the range of concentrations tested in the present study. Thus, suppression of GABA- and glycine-mediated synaptic transmission by M3G may explain the development of allodynia, hyperalgesia, seizures, and myoclonus that occur with high-dose opioid administration and may dictate the limiting dose of morphine that can be administered.

Although it is well established that DAMGO suppresses EPSCs in substantia gelatinosa neurons (Glaum et al., 1994; Kohno et al., 1999), its effect on IPSCs is controversial. In the substantia gelatinosa of the lumbar spinal cord, DAMGO reportedly did not affect inhibitory synaptic transmission (Kohno et al., 1999), whereas in trigeminal nucleus pars caudalis DAMGO, suppressed GABAergic and glycnergic IPSCs (Grudt and Henderson, 1998). We found that DAMGO suppresses IPSCs in lumbar substantia gelatinosa, which supports the findings of Grudt and Henderson (1998). One reason for the disparate findings may be the temperature at which the various studies were done. The work of Kohno et al. (1999) was done at 37°C, whereas our work and that of Grudt and Henderson (1998) were done at lower temperatures (24 or 30°C). If there is an increased safety factor for inhibitory synaptic transmission at higher temperatures, this may explain the insensitivity of IPSCs to DAMGO that was noted by Kohno et al. (1999). We also corroborated previous findings that N/OFQ selectively suppresses IPSCs in the substantia gelatinosa (Lai et al., 1997; Liebel et al., 1997; Zeilhofer et al., 2000; Ahmadi et al., 2001).

Because actions of M3G at μ-, δ-, and κ-opioid and ORL_1 receptors (Pasternak et al., 1987; Sullivan et al., 1989; Löser et al., 1996) have now been excluded, the receptor through which M3G exerts its effect remains to be determined. Interestingly, the selective presynaptic effect of M3G on IPSCs is similar to that of the recently identified neuropeptide, nocistatin. Nocistatin, like M3G, selectively suppresses IPSCs in the dorsal horn via a presynaptic mechanism (Zeilhofer et al., 2000) and also has pro-nociceptive actions in behavioral tests (Xu et al., 1999; Zeilhofer et al., 2000; Ahmadi et al.,

The failure of M3G to antagonize the effects of DAMGO and the lack of effect of M3G on evoked EPSCs is consistent with receptor binding studies, which show M3G has little or no affinity for the μ-opioid receptor (Pasternak et al., 1987; Sullivan et al., 1989; Lo et al., 1996). In addition, our findings agree with a previous electrophysiological study (Hewett et al., 1993), which indicated that M3G does not antagonize the antinociceptive actions of intrathecal morphine. Moreover, lack of antagonism of the actions of N/OFQ suggests that M3G also does not interact with the ORL_1 receptor in rat substantia gelatinosa.

By contrast, with its lack of effect on excitatory synaptic transmission, M3G produced a concentration-dependent suppression on inhibitory synaptic transmission. At a concentration of 100 μM, M3G reduced the amplitude of evoked IPSCs by approximately 45%. In the presence of TTX, M3G reduced the mIPSC frequency without affecting the amplitude distribution in all cells tested, suggesting the effect of M3G involved a presynaptic mechanism. If M3G had exerted an effect on postsynaptic GABA_A or glycine receptors, a change in mIPSC amplitude would likely have been observed. Similarly, a postsynaptic action of M3G would not account for the observed increase in the paired-pulse ratio. The effect on evoked responses in the superficial dorsal horn (Sullivan et al., 1989; Hewett et al., 1993)

Fig. 8. N/OFQ and DAMGO have different actions on inhibitory synaptic transmission in substantia gelatinosa neurons. IPSCs were evoked at a holding potential of 0 mV by focal stimulation in the presence of 50 μM AP5 and 10 μM CNQX. A, the top panel shows averaged traces (n = 3) of evoked IPSCs before, during, and after application of 1 μM N/OFQ. Note N/OFQ does not affect the amplitude of the evoked IPSC. B, averaged traces (n = 3) of evoked IPSCs before, during, and after application of 1 μM DAMGO. Note that unlike N/OFQ, DAMGO reduces the amplitude of the evoked IPSC. C, time course of the amplitude of evoked IPSCs in the presence of DAMGO from the cell illustrated in B. D, summary histogram comparing the effects of N/OFQ (1 μM, n = 5), DAMGO, (1 μM, n = 6), and M3G (100 μM, n = 10) on IPSC amplitude.

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Our observed effects may be especially relevant to palliative care situations, where heroic doses of morphine (up to 20 g/day) are required to produce analgesia in tolerant individuals (Hagen and Swanson, 1997; Sjogren et al., 1998). In humans, intrathecal injection of 1100 mg of morphine results in an accumulation of ~3 μM M3G in CSF (Goucke et al., 1994). By extrapolation, the concentration of M3G in the CSF of a palliative care patient who had received 20 g of morphine within a day would approach 60 μM (Hagen and Swanson, 1997; Sjogren et al., 1998). This falls within the range of concentrations tested in the present study. Thus, suppression of GABA- and glycine-mediated synaptic transmission by M3G may explain the development of allodynia, hyperalgesia, seizures, and myoclonus that occur with high-dose opioid administration and may dictate the limiting dose of morphine that can be administered.

Although it is well established that DAMGO suppresses EPSCs in substantia gelatinosa neurons (Glaum et al., 1994; Kohno et al., 1999), its effect on IPSCs is controversial. In the substantia gelatinosa of the lumbar spinal cord, DAMGO reportedly did not affect inhibitory synaptic transmission (Kohno et al., 1999), whereas in trigeminal nucleus pars caudalis DAMGO, suppressed GABAergic and glycnergic IPSCs (Grudt and Henderson, 1998). We found that DAMGO suppresses IPSCs in lumbar substantia gelatinosa, which supports the findings of Grudt and Henderson (1998). One reason for the disparate findings may be the temperature at which the various studies were done. The work of Kohno et al. (1999) was done at 37°C, whereas our work and that of Grudt and Henderson (1998) were done at lower temperatures (24 or 30°C). If there is an increased safety factor for inhibitory synaptic transmission at higher temperatures, this may explain the insensitivity of IPSCs to DAMGO that was noted by Kohno et al. (1999). We also corroborated previous findings that N/OFQ selectively suppresses IPSCs in the substantia gelatinosa (Lai et al., 1997; Liebel et al., 1997; Zeilhofer et al., 2000; Ahmadi et al., 2001).

Because actions of M3G at μ-, δ-, and κ-opioid and ORL_1 receptors (Pasternak et al., 1987; Sullivan et al., 1989; Löser et al., 1996) have now been excluded, the receptor through which M3G exerts its effect remains to be determined. Interestingly, the selective presynaptic effect of M3G on IPSCs is similar to that of the recently identified neuropeptide, nocistatin. Nocistatin, like M3G, selectively suppresses IPSCs in the dorsal horn via a presynaptic mechanism (Zeilhofer et al., 2000) and also has pro-nociceptive actions in behavioral tests (Xu et al., 1999; Zeilhofer et al., 2000; Ahmadi et al.,
2001). It is thus possible that M3G interacts with the nociceptin receptor, but until this receptor is better characterized and antagonists are developed, this possibility remains to be investigated.

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References


