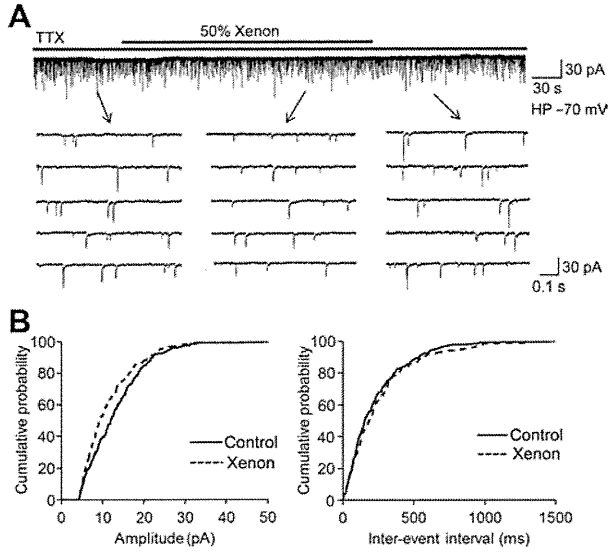

Fig. 6. Xenon decreased the mean amplitudes but not the mean frequency of miniature excitatory postsynaptic currents (EPSCs) in the spinal lamina IX neurons. Continuous chart recording of miniature EPSCs at −70 mV in the presence of Tetrodotoxin (TTX) (1 μM) during the action of xenon (*upper trace*). Five consecutive traces of miniature EPSCs are shown on an expanded time scale (*lower traces*) (*A*). Cumulative distributions of the amplitude (*left*) and interevent interval (*right*) of miniature EPSCs before (*continuous line*) and during (*dotted line*) the action of xenon. Xenon had little effect on the distribution of the interevent interval but shifted the distribution to a smaller amplitude, as determined using the Kolmogorov-Smirnov test (*B*). HP = holding potential.

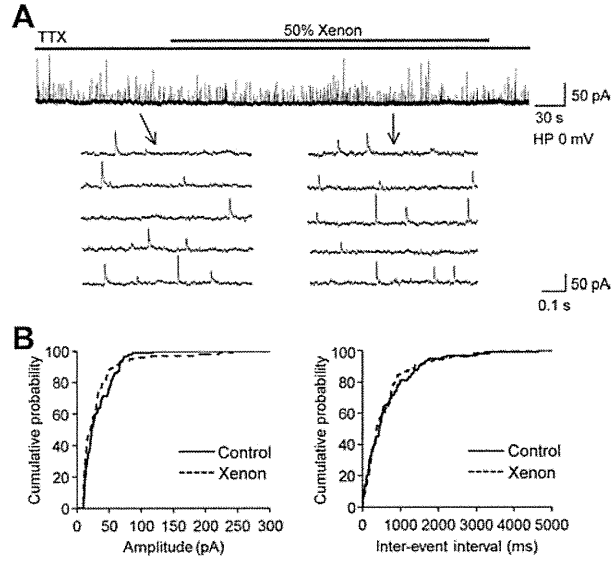

Fig. 7. Xenon affected neither the mean amplitudes nor the mean frequency of miniature inhibitory postsynaptic currents (IPSCs) in the spinal lamina IX neurons. Continuous chart recording of miniature IPSCs at 0 mV in the presence of Tetrodotoxin (TTX) (1 μM) during the action of xenon (*upper trace*) (*A*). Five consecutive traces of miniature IPSCs are shown on an expanded time scale (*lower traces*). Cumulative distributions of the amplitude (*left*) and interevent interval (*right*) of miniature IPSCs before (*continuous line*) and during (*dotted line*) the action of xenon. Xenon had little effect on the distributions both of the amplitude and interevent interval, as determined using the Kolmogorov-Smirnov test (*B*). HP = holding potential.

pressed ubiquitously in the entire spinal cord, including the spinal ventral horn motoneurons and the spinal dorsal horn neurons.[17–20] The functional properties of NMDA receptors are determined by the NR2 subunit composition (NR2A–2D).[20–22] The NR3 subunits (NR3A and 3B) occasionally are expressed in addition to NR1 and NR2 subunits and functionally suppress NMDA receptors.[23,24] The NR2C and NR2D subunits are expressed weakly, but the NR2A and NR2B subunits are not identified in substantia gelatinosa neurons of the adult rat lumbar spinal cord.[18] We reported previously that 50% xenon depressed NMDA receptor-mediated glutamatergic excitatory transmission *via* a postsynaptic mechanism in adult substantia gelatinosa neurons.[8] By contrast, the NR2A subunit is expressed predominantly in neonatal motoneurons.[19] However, around postnatal day 10–14, the NR2A subunit decreases, and the major regulatory subunit of the NMDA receptors switches to the NR3B subunit; the NR3B subunit is maintained until the adult stage.[19] The expression of the NR3B subunit is restricted to somatic motoneurons in the adult brainstem and spinal cord.[19] Considering the neonatal age of rats in the current study, these reports suggest that we may have examined the effects of xenon mainly on the NR2A subunit; the lack of NMDA receptor response to xenon may be caused by the lack of response of the NR2A subunit to xenon. In short, the subunits of the NMDA receptors expressed in neonatal motoneurons and in adult substantia gelatinosa neurons are different. This different composition of NMDA receptors may underlie the variability among tissues in the effects of xenon on NMDA receptor-mediated synaptic transmission.

In our current study, we were confronted with the issue of xenon concentration. At the beginning, a single concentration of 50% xenon was used in our experiments for technical and financial reasons. The MAC of xenon is estimated to be 86–161% in rats[25,26]; thus, 50% xenon is calculated to be 0.31–0.58 rat MAC. Xenon is known to have analgesic properties even at subanesthetic (0.3 MAC) concentrations in rats[27] and humans.[28,29] Although the concentration of dissolved xenon could not be measured, we calculated the concentration of dissolved 50% xenon at approximately 1.9 mM according to previous studies.[4–6,30,31] A previous study showed that 1.9 mM dissolved xenon depressed the NR2A-containing NMDA receptors in the amygdala.[32] In addition, we previously demonstrated that 50% xenon depressed NMDA receptor-mediated glutamatergic excitatory transmission in adult substantia gelatinosa neurons.[8] Therefore, we concluded that this 50% xenon concentration was sufficient to modulate synaptic transmission in the spinal lamina IX neurons. Nevertheless, 50% xenon did not affect NMDA

receptor-mediated synaptic transmission in our current investigation. However, xenon may suppress NMDA receptor-mediated synaptic transmission or may produce some other effects at higher concentration. These effects would be more critical to immobilization than the effects on AMPA receptor-mediated synaptic transmission observed in this study. Thus, we also examined the effects of 75% xenon on AMPA- and NMDA-induced currents. Seventy-five percent xenon reversibly reduced the peak amplitudes and the integrated area of the AMPA-induced currents. However, 75% xenon did not change the peak amplitudes or the integrated area of NMDA-induced currents. Considering the maintenance of physiologic concentration of oxygen and carbon dioxide, 75% xenon is the maximum concentration in our current investigation. Nevertheless, NMDA-induced currents were not affected by 75% xenon.

Non-NMDA glutamate receptors include both AMPA receptors and kainate receptors. Neonatal rat motoneurons also express kainate receptors.[33] However, most of the glutamatergic non-NMDA receptor-mediated synaptic transmissions in neonatal rodent motoneurons are mediated *via* AMPA receptors.[34–36] Therefore, we interpreted glutamatergic non-NMDA receptor-mediated synaptic transmission as AMPA receptor-mediated synaptic transmission in this study.

The effects of 50% xenon on AMPA receptor-mediated synaptic transmission in the spinal lamina IX neurons were less than those in the spinal dorsal horn neurons[8]; however, several previous reports have indicated that the dorsal horn plays a lesser role in immobility and have suggested that the ventral horn is the site of anesthetic actions.[37–39] These results might also suggest that xenon acts differently than other anesthetics by acting predominantly on the spinal dorsal horn neurons, rather than on the spinal ventral horn motoneurons.

In a previous study, Cheng and Kendig[35] investigated the effects of the inhalational anesthetic enflurane on synaptic transmission in spinal ventral horn neurons. Electrically evoked EPSCs were elicited by electrical stimuli applied to the dorsal root, and the researchers concluded that enflurane depressed glutamatergic excitatory synaptic transmission in motoneurons.[35] However, the inhalational anesthetic isoflurane[40] and nitrous oxide[41] affected synaptic transmission in substantia gelatinosa neurons of the adult rat spinal cord. It was also reported that isoflurane affected electrically evoked EPSCs in substantia gelatinosa neurons of the immature rat spinal cord.[42] In addition, xenon inhibited AMPA and NMDA receptor-mediated excitatory synaptic transmission in substantia gelatinosa neurons of the adult rat spinal cord.[7,8] These results suggest that the electrically evoked postsynaptic currents may be affected in the substantia gelatinosa before reaching the spinal lamina IX neurons when the dorsal root or root entry zone is stimulated. Thus, in the current study, we elicited electrically evoked EPSCs by focal stimulation of the deep dorsal horn. Despite using this method, we could not completely exclude the possibility that deep dorsal horn stimulation might evoke monosynaptic responses such as Ia inputs that may not be relevant to the MAC determination.

The ventral interneuronal networks are also important for generating motor responses to noxious stimuli and determining the MAC.[37,39] The importance of ventral interneurons is supported by the evidence that spinal sensory neurons and motoneurons are relatively resistant to anesthetics, despite showing some individually depressive effects.[43]

To identify large spinal lamina IX neurons under an infrared-differential interference contrast microscope, we excluded older rats, whose spinal lamina IX neurons were impossible to identify because of highly developed fibrous tissue. However, the major regulatory subunit of the NMDA receptors switches from NR2A to NR3B in motoneurons around postnatal day 10–14,[19] and this switch of NMDA receptor subunits indicates a possibility that xenon may depress NMDA receptor-mediated synaptic transmission in adult motoneurons. In contrast, another study showed that the sensitivities of NMDA receptors to Mg^{2+}, isoflurane, ketamine, nitrous oxide, and ethanol were not altered by the NR3B subunit, although the NR3B subunit prominently reduced the amplitude of NMDA currents.[44] These results indicate that NMDA receptor-mediated synaptic transmission may not be affected by xenon in adult motoneurons. In any case, additional studies are required using identified spinal motoneurons in the adult rat.

In conclusion, we investigated the effects of xenon on the spinal lamina IX neurons as one of the components of the spinal reflex pathway and clarified that xenon inhibited AMPA receptor-mediated glutamatergic excitatory synaptic transmission in the spinal lamina IX neurons *via* a postsynaptic mechanism. These results indicate that the suppression of neuronal activity in the spinal ventral horn and substantia gelatinosa neurons might contribute to immobility and could account for the mechanism of xenon as an anesthetic for the prevention of movement in response to noxious stimuli and to determine the MAC. However, the modest effects of xenon on AMPA receptors and the lack of effect on the NMDA receptors might mean that the spinal ventral horn neurons are not a likely site for the immobilizing action of xenon; additional investigation of ventral interneurons existing in the lamina VII and VIII would help resolve these questions.

References

1. Preckel B, Schlack W: Inert gases as the future inhalational anaesthetics? Best Pract Res Clin Anaesthesiol 2005; 19: 365–79
2. Derwall M, Coburn M, Rex S, Hein M, Rossaint R, Fries M: Xenon: Recent developments and future perspectives. Minerva Anestesiol 2009; 75:37–45

3. Preckel B, Weber NC, Sanders RD, Maze M, Schlack W: Molecular mechanisms transducing the anesthetic, analgesic, and organ-protective actions of xenon. ANESTHESIOLOGY 2006; 105:187-97
4. de Sousa SL, Dickinson R, Lieb WR, Franks NP: Contrasting synaptic actions of the inhalational general anesthetics isoflurane and xenon. ANESTHESIOLOGY 2000; 92:1055-66
5. Dinse A, Föhr KJ, Georgieff M, Beyer C, Bulling A, Weigt HU: Xenon reduces glutamate-, AMPA-, and kainate-induced membrane currents in cortical neurones. Br J Anaesth 2005; 94:479-85
6. Haseneder R, Kratzer S, Kochs E, Eckle VS, Zieglgänsberger W, Rammes G: Xenon reduces *N*-methyl-D-aspartate and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor-mediated synaptic transmission in the amygdala. ANESTHESIOLOGY 2008; 109:998-1006
7. Haseneder R, Kratzer S, Kochs E, Mattusch C, Eder M, Rammes G: Xenon attenuates excitatory synaptic transmission in the rodent prefrontal cortex and spinal cord dorsal horn. ANESTHESIOLOGY 2009; 111:1297-307
8. Georgiev SK, Furue H, Baba H, Kohno T: Xenon inhibits excitatory but not inhibitory transmission in rat spinal cord dorsal horn neurons. Mol Pain 2010; 6:25
9. Eger EI 2nd, Saidman LJ, Brandstater B: Minimum alveolar anesthetic concentration: A standard of anesthetic potency. ANESTHESIOLOGY 1965; 26:756-63
10. Antognini JF, Schwartz K: Exaggerated anesthetic requirements in the preferentially anesthetized brain. ANESTHESIOLOGY 1993; 79:1244-9
11. Rampil IJ, Mason P, Singh H: Anesthetic potency (MAC) is independent of forebrain structures in the rat. ANESTHESIOLOGY 1993; 78:707-12
12. Collins JG, Kendig JJ, Mason P: Anesthetic actions within the spinal cord: Contributions to the state of general anesthesia. Trends Neurosci 1995; 18:549-53
13. Honda H, Baba H, Kohno T: Electrophysiological analysis of vulnerability to experimental ischemia in neonatal rat spinal ventral horn neurons. Neurosci Lett 2011; 494:161-4
14. Wang MY, Rampil IJ, Kendig JJ: Ethanol directly depresses AMPA and NMDA glutamate currents in spinal cord motor neurons independent of actions on GABAA or glycine receptors. J Pharmacol Exp Ther 1999; 290:362-7
15. Franks NP, Dickinson R, de Sousa SL, Hall AC, Lieb WR: How does xenon produce anaesthesia? Nature 1998; 396:324
16. Yamakura T, Harris RA: Effects of gaseous anesthetics nitrous oxide and xenon on ligand-gated ion channels. Comparison with isoflurane and ethanol. ANESTHESIOLOGY 2000; 93:1095-101
17. Kutsuwada T, Kashiwabuchi N, Mori H, Sakimura K, Kushiya E, Araki K, Meguro H, Masaki H, Kumanishi T, Arakawa M, Mishina M: Molecular diversity of the NMDA receptor channel. Nature 1992; 358:36-41
18. Tölle TR, Berthele A, Zieglgänsberger W, Seeburg PH, Wisden W: The differential expression of 16 NMDA and non-NMDA receptor subunits in the rat spinal cord and in periaqueductal gray. J Neurosci 1993; 13:5009-28
19. Fukaya M, Hayashi Y, Watanabe M: NR2 to NR3B subunit switchover of NMDA receptors in early postnatal motoneurons. Eur J Neurosci 2005; 21:1432-6
20. Ishii T, Moriyoshi K, Sugihara H, Sakurada K, Kadotani H, Yokoi M, Akazawa C, Shigemoto R, Mizuno N, Masu M: Molecular characterization of the family of the *N*-methyl-D-aspartate receptor subunits. J Biol Chem 1993; 268:2836-43
21. Monyer H, Burnashev N, Laurie DJ, Sakmann B, Seeburg PH: Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. Neuron 1994; 12:529-40
22. Yamakura T, Shimoji K: Subunit- and site-specific pharmacology of the NMDA receptor channel. Prog Neurobiol 1999; 59:279-98
23. Nishi M, Hinds H, Lu HP, Kawata M, Hayashi Y: Motoneuron-specific expression of NR3B, a novel NMDA-type glutamate receptor subunit that works in a dominant-negative manner. J Neurosci 2001; 21:RC185
24. Sucher NJ, Akbarian S, Chi CL, Leclerc CL, Awobuluyi M, Deitcher DL, Wu MK, Yuan JP, Jones EG, Lipton SA: Developmental and regional expression pattern of a novel NMDA receptor-like subunit (NMDAR-L) in the rodent brain. J Neurosci 1995; 15:6509-20
25. David HN, Leveille F, Chazalviel L, MacKenzie ET, Buisson A, Lemaire M, Abraini JH: Reduction of ischemic brain damage by nitrous oxide and xenon. J Cereb Blood Flow Metab 2003; 23:1168-73
26. Koblin DD, Fang Z, Eger EI 2nd, Laster MJ, Gong D, Ionescu P, Halsey MJ, Trudell JR: Minimum alveolar concentrations of noble gases, nitrogen, and sulfur hexafluoride in rats: Helium and neon as nonimmobilizers (nonanesthetics). Anesth Analg 1998; 87:419-24
27. Watanabe I, Takenoshita M, Sawada T, Uchida I, Mashimo T: Xenon suppresses nociceptive reflex in newborn rat spinal cord *in vitro*: Comparison with nitrous oxide. Eur J Pharmacol 2004; 496:71-6
28. Yagi M, Mashimo T, Kawaguchi T, Yoshiya I: Analgesic and hypnotic effects of subanaesthetic concentrations of xenon in human volunteers: Comparison with nitrous oxide. Br J Anaesth 1995; 74:670-3
29. Petersen-Felix S, Luginbühl M, Schnider TW, Curatolo M, Arendt-Nielsen L, Zbinden AM: Comparison of the analgesic potency of xenon and nitrous oxide in humans evaluated by experimental pain. Br J Anaesth 1998; 81:742-7
30. Hapfelmeier G, Zieglgänsberger W, Haseneder R, Schneck H, Kochs E: Nitrous oxide and xenon increase the efficacy of GABA at recombinant mammalian GABA(A) receptors. Anesth Analg 2000; 91:1542-9
31. Plested AJ, Wildman SS, Lieb WR, Franks NP: Determinants of the sensitivity of AMPA receptors to xenon. ANESTHESIOLOGY 2004; 100:347-58
32. Haseneder R, Kratzer S, Kochs E, Höfelmann D, Auberson Y, Eder M, Rammes G: The xenon-mediated antagonism against the NMDA receptor is non-selective for receptors containing either NR2A or NR2B subunits in the mouse amygdala. Eur J Pharmacol 2009; 619:33-7
33. Stegenga SL, Kalb RG: Developmental regulation of *N*-methyl-D-aspartate- and kainate-type glutamate receptor expression in the rat spinal cord. Neuroscience 2001; 105:499-507
34. Cheng G, Kendig JJ: Enflurane decreases glutamate neurotransmission to spinal cord motor neurons by both pre- and postsynaptic actions. Anesth Analg 2003; 96:1354-9
35. Cheng G, Kendig JJ: Enflurane directly depresses glutamate AMPA and NMDA currents in mouse spinal cord motor neurons independent of actions on GABAA or glycine receptors. ANESTHESIOLOGY 2000; 93:1075-84
36. Li HF, Wang MY, Knape J, Kendig JJ: Ethanol tachyphylaxis in spinal cord motorneurons: Role of metabotropic glutamate receptors. Br J Pharmacol 2003; 138:1417-24
37. Jinks SL, Martin JT, Carstens E, Jung SW, Antognini JF: Peri-MAC depression of a nociceptive withdrawal reflex is accompanied by reduced dorsal horn activity with halothane but not isoflurane. ANESTHESIOLOGY 2003; 98:1128-38
38. Kim J, Yao A, Atherley R, Carstens E, Jinks SL, Antognini JF: Neurons in the ventral spinal cord are more depressed by isoflurane, halothane, and propofol than are neurons in the dorsal spinal cord. Anesth Analg 2007; 105:1020-6
39. Mitsuyo T, Dutton RC, Antognini JF, Carstens E: The differential effects of halothane and isoflurane on windup of

dorsal horn neurons selected in unanesthetized decerebrated rats. Anesth Analg 2006; 103:753–60

40. Wakai A, Kohno T, Yamakura T, Okamoto M, Ataka T, Baba H: Action of isoflurane on the substantia gelatinosa neurons of the adult rat spinal cord. ANESTHESIOLOGY 2005; 102: 379–86
41. Georgiev SK, Kohno T, Ikoma M, Yamakura T, Baba H: Nitrous oxide inhibits glutamatergic transmission in spinal dorsal horn neurons. Pain 2008; 134:24–31
42. Haseneder R, Kurz J, Dodt HU, Kochs E, Zieglgänsberger W, Scheller M, Rammes G, Hapfelmeier G: Isoflurane reduces glutamatergic transmission in neurons in the spinal cord superficial dorsal horn: Evidence for a presynaptic site of an analgesic action. Anesth Analg 2004; 98:1718–23
43. Eger EI 2nd, Raines DE, Shafer SL, Hemmings HC Jr, Sonner JM: Is a new paradigm needed to explain how inhaled anesthetics produce immobility? Anesth Analg 2008; 107: 832–48
44. Yamakura T, Askalany AR, Petrenko AB, Kohno T, Baba H, Sakimura K: The NR3B subunit does not alter the anesthetic sensitivities of recombinant *N*-methyl-D-aspartate receptors. Anesth Analg 2005; 100:1687–92

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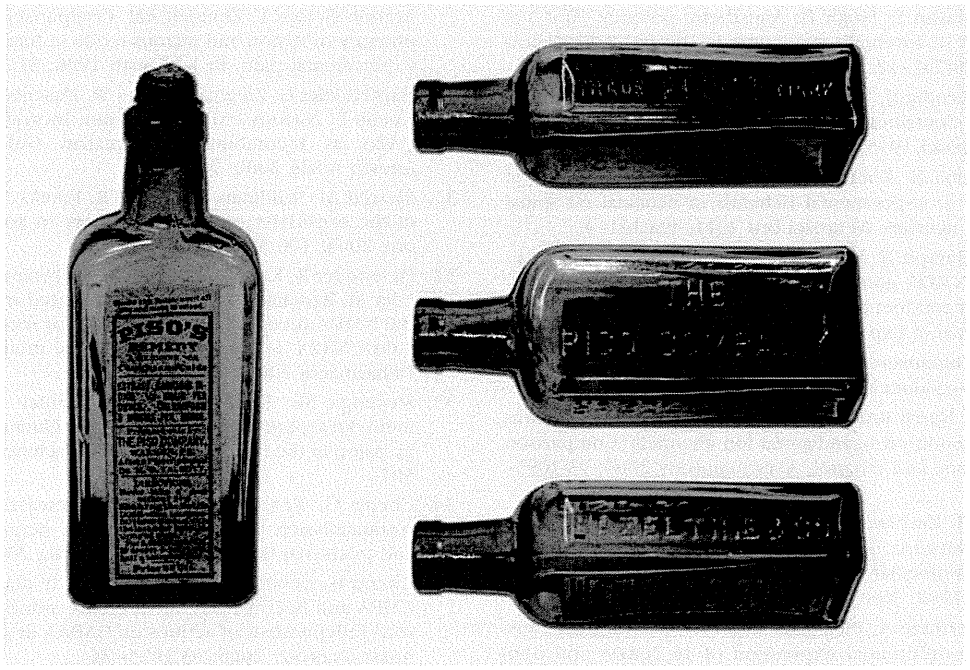
Weeding Out Piso's Cure



Originally formulated in 1864 with opium and morphine as "Piso's Consumption Cure," this glorified cough syrup shed its opiates by the 1880s. By 1904 "Piso's Cure" was renamed "Piso's Remedy for Coughs and Colds" (*left*). In May of 1909 the American Medical Association blacklisted "Piso's Cure" as a "habit-forming drug." Perhaps that is understandable, since "piso" (Spanish for "floor") was a marijuana-chloroform elixir which "floored" its imbibers. (Copyright © the American Society of Anesthesiologists, Inc.)

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The Mu Opioid Receptor Modulates Neurotransmission in the Rat Spinal Ventral Horn

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BACKGROUND: Opioids inhibit excitatory neurotransmission and produce antinociception through μ opioid receptors (MORs). Although MORs are expressed in the spinal ventral horn, their functions and effects are largely unknown. Therefore, we examined the neuromodulatory effects of μ opioids in spinal lamina IX neurons at the cellular level.
METHODS: The effects of the selective μ agonist [D-Ala^2,-*N*-Me-Phe^4, Gly^5-ol]enkephalin (DAMGO) on synaptic transmission were examined in spinal lamina IX neurons of neonatal rats using the whole-cell patch-clamp technique.
RESULTS: DAMGO produced outward currents in 56% of the lamina IX neurons recorded, with a 50% effective concentration of 0.1 μM. Analysis of the current-voltage relationship revealed a reversal potential of approximately −86 mV. These currents were not blocked by tetrodotoxin but were inhibited by Ba^{2+} or a selective μ antagonist. Moreover, the currents were suppressed by the addition of Cs^+ and tetraethylammonium or guanosine 5′-[β-thio]diphosphate trilithium salt to the pipette solution. In addition, DAMGO decreased the frequency of spontaneous excitatory and inhibitory postsynaptic currents, and these effects were unaltered by treatment with tetrodotoxin.
CONCLUSION: Our results suggest that DAMGO hyperpolarizes spinal lamina IX neurons by G protein–mediated activation of K^+ channels after activation of MORs. Furthermore, activation of MORs on presynaptic terminals reduces both excitatory and inhibitory transmitter release. Although traditionally opioids are not thought to affect motor function, the present study documents neuromodulatory effects of μ opioids in spinal lamina IX neurons, suggesting that MORs can influence motor activity. (Anesth Analg 2012;115:703–12)

Opioids are important neuromodulators in the central nervous system and are widely used in clinical settings. Opioids primarily exert inhibitory effects that include postsynaptic hyperpolarization through the opening of several types of K^+ channels, leading to a reduction in neuronal excitability and presynaptic inhibition of excitatory neurotransmitter release.[1,2] Indeed, the powerful analgesic effect of μ opioid receptor (MOR) agonists is partially mediated by their suppressive actions in the spinal dorsal horn.[3–5] The ventral horn of the spinal cord contains motoneuron pools, which are known as lamina IX,[6] and although MORs are expressed in the spinal ventral horn,[7–9] little is known about opioid effects on motoneurons in this region.

Traditionally, opioids are not thought to affect motor function. However, several lines of evidence suggest that opioids can affect spinal motoneuronal excitability. For example, multiple groups have reported that μ opioids decreased the amplitude of motor evoked potentials while increasing latency.[10–12] Fernandez-Galinski et al.[10] proposed that activation of MORs in the ventral horn could delay depolarization of spinal motoneurons and thereby prolong latency. In addition, they inferred that μ opioids inhibited the excitatory interneurons that depolarize spinal motoneurons. Conversely, it has also been reported that opioids have excitatory effects in some central nervous system regions.[13,14] In this regard, MOR agonists were shown to cause spastic paraplegia in the setting of prior ischemic injury,[15,16] and the MOR antagonist naloxone ameliorated motor deficits after spinal cord ischemia.[17] Given that cell death after ischemic injury is thought to result from overexcitation of the neuronal membrane,[18] the effects of MOR activation in this system may include depolarization of spinal motoneurons.

Despite these indications that μ opioids act as important neuromodulators in the spinal ventral horn, the cellular effects of MOR activation in this region have not been clarified. Therefore, the aim of the present study was to examine the neuromodulatory effects of μ opioids in spinal ventral horn neurons at the cellular level. Specifically, we examined the effects of a selective MOR agonist, [D-Ala^2, -*N*-Me-Phe^4, Gly^5-ol]enkephalin (DAMGO), in spinal lamina IX neurons of rats, using the whole-cell patch-clamp technique. Our results provide new evidence that MOR activation modulates neurotransmission in the spinal ventral horn.

METHODS

Preparation of Spinal Cord Slices

All experimental procedures involving the use of animals were approved by the Animal Care and Use Committee at Niigata University Graduate School of Medical and Dental

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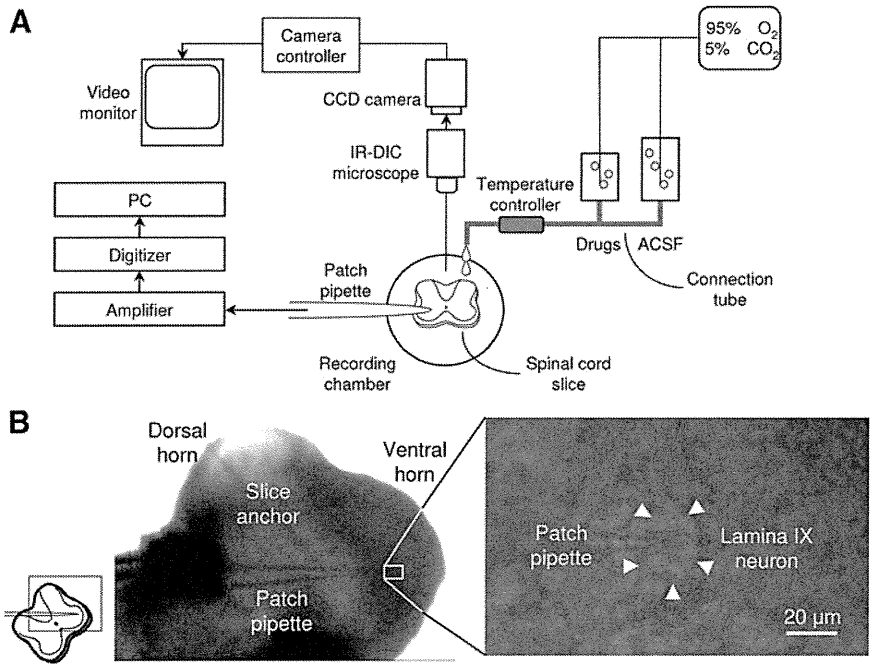

Figure 1. Schematic representation of the experimental setup. A, The spinal cord slice was placed in a recording chamber and visualized with an upright microscope equipped with an infrared–differential interference contrast (IR-DIC) system. Drugs were applied by superfusion without alteration of the perfusion rate or temperature. ACSF = artificial cerebrospinal fluid; CCD = charge coupled device; PC = personal computer. B, Spinal cord slice preparation viewed under a 5× objective lens (left) and 40× objective lens (right) of an IR-DIC microscope. The ventral horn neuron with a large soma was identified as a spinal lamina IX neuron (arrowheads).

Sciences (Niigata, Japan). Slices of rat spinal cord were prepared as previously described.[19] In brief, neonatal Wistar rats (8–12 days old) were anesthetized with urethane (1.2–1.5 g · kg^{-1}, intraperitoneally). After dorsal laminectomy, the lumbosacral segment of the spinal cord was removed, and the animal was immediately killed by exsanguination. The spinal cord was immersed in ice-cold artificial cerebrospinal fluid (ACSF; 117 mM NaCl, 3.6 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 1.2 mM $NaH_2orally_4$, 25 mM $NaHCO_3$, and 11.5 mM D-glucose; pH 7.4) equilibrated with a gas mixture of 95% O_2 and 5% CO_2. The spinal cord was cut into 500-μm-thick transverse slices using a microslicer (DTK-1500; Dosaka, Kyoto, Japan). Slices were transferred to a recording chamber and placed on the stage of an upright microscope equipped with an infrared–differential interference contrast (IR-DIC) system (E600FN; Nikon, Tokyo, Japan). The slices were superfused at 5 mL · min^{-1} with ACSF solution and were maintained at 36°C using a temperature controller (TC-324B; Warner Instruments, Hamden, CT) for at least 1 hour before recordings (Fig. 1A).

Patch-Clamp Recordings from Spinal Lamina IX Neurons

Lamina regions were identified under low magnification (5× objective lens), and individual neurons were detected using a 40× objective lens under an IR-DIC microscope and monitored using a charge coupled device camera (C2400-79H; Hamamatsu Photonics, Hamamatsu, Japan) and a video monitor (Fig. 1A). The size of each neuron was calculated from the arithmetic mean diameter of the long and short axes of the soma intersecting at right angles. Whole-cell patch-clamp recordings were made from large lamina IX neurons (size, 15–25 μm), most frequently seen in the ventral lateral or ventral medial area (Fig. 1B).[20,21] In a previous study, these neurons were identified as motoneurons by fluorescence labeling with Evans blue dye injected into the rat hindlimb the day before death.[22] After the whole-cell configuration was established, voltage-clamped neurons were held at −70 mV or 0 mV. Whole-cell patch pipettes were constructed from borosilicate glass capillaries (1.5 mm outer diameter; World Precision Instruments, Sarasota, FL). The resistance of a typical patch pipette was 4 to 8 MΩ when filled with internal solution. Two internal patch pipette solutions were used, with compositions as follows: (1) a potassium gluconate-based solution containing 135 mM potassium gluconate, 5 mM KCl, 0.5 mM $CaCl_2$, 2 mM $MgCl_2$, 5 mM EGTA, 5 mM HEPES, and 5 mM ATP-Mg (pH 7.2); and (2) a cesium sulfate-based solution containing 110 mM Cs_2SO_4, 5 mM tetraethylammonium (TEA), 0.5 mM $CaCl_2$, 2 mM $MgCl_2$, 5 mM EGTA, 5 mM HEPES, 5 mM ATP-Mg, and 2 mM guanosine 5′-[β-thio]diphosphate trilithium salt (GDP-β-S) (pH 7.2). The latter solution was used to abolish the postsynaptic effects of DAMGO. Signals were amplified by an Axopatch 200B amplifier (Molecular Devices, Union City, CA) and filtered at 2 kHz and digitized at 5 kHz. Data were stored and analyzed using the pCLAMP 9.1 data acquisition program (Molecular Devices). Voltage ramps (duration: 400 milliseconds) from the −70 mV holding potential (approximately −110 to −50 mV) were used to examine current-voltage relationships.

Drug Application

DAMGO, D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2 (CTAP), and barium chloride dehydrate (Ba^{2+}) were from Sigma-Aldrich (St. Louis, MO). Tetrodotoxin (TTX) was from Wako (Osaka, Japan). All drugs were first dissolved in distilled water at 1000 times the working concentration, then diluted to the working concentration in ACSF solution immediately before use. Drugs were applied by switching the perfusion solution via a 3-way stopcock without a change in the perfusion rate or temperature.

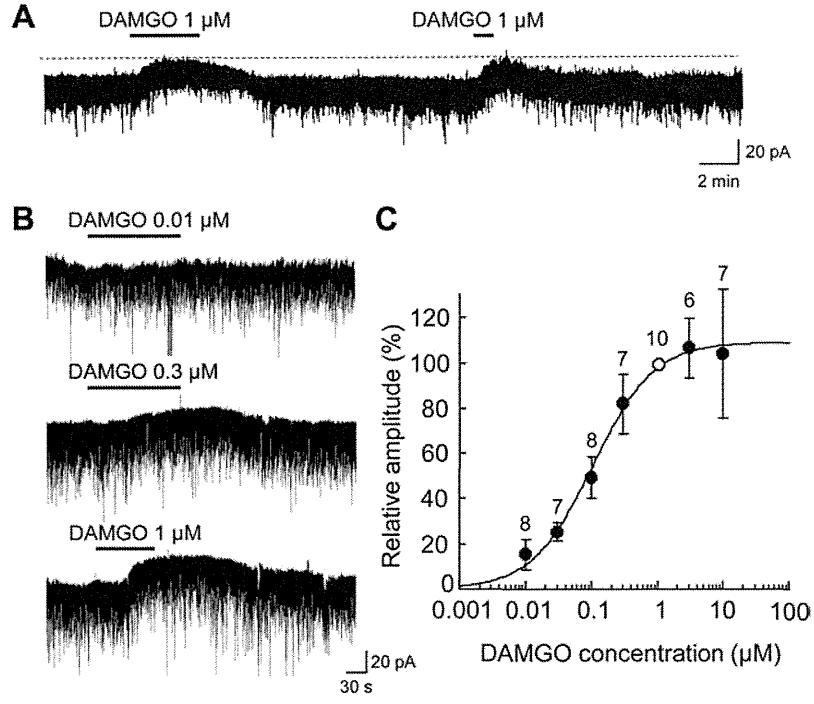

Figure 2. [D-Ala^2-*N*-Me-Phe^4, Gly^5-ol]enkephalin (DAMGO) induced outward currents in lamina IX neurons in a dose-dependent manner. A, Representative current traces produced by DAMGO (1 μM). After washout, the amplitude gradually returned to the previous level, and subsequent application of DAMGO elicited the same reaction. In this figure and subsequent figures, the horizontal bars above the chart recordings indicate the duration of drug superfusion. B, Amplitude of the DAMGO-induced outward currents was increased with increasing concentrations of DAMGO (0.01, 0.3, and 1 μM) in the same neuron. C, Peak amplitudes (closed circles) of the outward currents elicited by DAMGO at various concentrations relative to those at 1 μM (an open circle), plotted against the logarithm of the concentration. We tested multiple concentrations including 1 μM DAMGO on each neuron. Each point with vertical bars represents the mean value and SEM. The figure above each point denotes the number of neurons examined. The continuous curve was drawn according to the Hill plot, with a 50% effective concentration of 0.1 μM (95% confidence interval, 0.09–0.12 μM) and a Hill coefficient of 0.95 (95% confidence interval, 0.84–1.05). Holding potential = −70 mV.

Data Analysis

The continuous curve for the concentration-response relationship of DAMGO was drawn according to the Hill equation:

$$y = \frac{y_{max}x^n}{k^n + x^n}$$

where x is the DAMGO concentration, y is the relative amplitude of DAMGO-induced current (%), and y_{max} is the maximal value of y. The term k is the 50% effective concentration (EC_{50}) (μM), and n is the slope of the curve (Hill coefficient). Numerical data are expressed as mean ± SEM. Statistical significance was defined as $P < 0.05$. Student paired *t* test or the Kolmogorov-Smirnov test was used as indicated for statistical analyses. For electrophysiological data, *n* refers to the number of neurons studied.

RESULTS

Whole-cell recordings could be obtained from slices maintained in vitro for >12 hours, and stable recordings were made from single neurons for up to 4 hours. The data presented herein were obtained from 184 lamina IX neurons. All neurons recorded exhibited spontaneous excitatory postsynaptic currents (sEPSCs) at the holding potential of −70 mV. However, when the potential was held at 0 mV, the neurons exhibited spontaneous inhibitory postsynaptic currents (sIPSCs).

Postsynaptic Actions of DAMGO in Lamina IX Neurons

Bath-applied DAMGO (1 μM) induced an outward current in 34 (56%) of 61 neurons; the average peak amplitude was 35 ± 3 pA at −70 mV. After DAMGO washout, the outward current amplitude gradually returned to control level in approximately 6 minutes, and subsequent application of DAMGO produced the same response (Fig. 2A). Therefore, in this study, the time interval between DAMGO applications was at least 10 minutes. The DAMGO-induced outward current increased in peak amplitude with increasing concentrations (Fig. 2B). Figure 2C shows the dose-response curve for the DAMGO-induced currents. Analysis of the curve based on the Hill plot indicated that the EC_{50} was 0.10 μM with a Hill coefficient of 0.95.

To confirm that the DAMGO-induced currents were postsynaptic effects, we examined the currents in the presence of TTX to remove any possible influence of MOR on presynaptic neurons. TTX (0.5 μM) had no significant effect on the amplitude of DAMGO-induced currents (101% ± 3% of control, $n = 6$; $P = 0.78$; Fig. 3, A and C). However, DAMGO-induced currents were attenuated by the selective MOR antagonist CTAP (5% ± 3% of control, $n = 5$; $P < 0.01$; Fig. 3, B and C). These results suggested that the DAMGO-induced current was mediated by postsynaptic MOR.

We next investigated the nature of the channels that mediate the DAMGO-induced outward current by evaluating membrane currents in response to voltage pulses in the absence and presence of the DAMGO-induced current (Fig. 4A). Figure 4B illustrates the relationship between the step voltage and steady current at the end of the pulse obtained in the absence (open triangles) and presence (closed triangles) of the DAMGO-induced current. The net DAMGO-induced current (open circles), which was calculated from the difference between the 2 currents, exhibited a clear reversal. The average reversal potential was −86 ± 3 mV ($n = 8$), which was slightly different from the equilibrium potential for K^+ (−97 mV) as calculated from the Nernst equation using the K^+ concentrations ($[K^+]_o$,

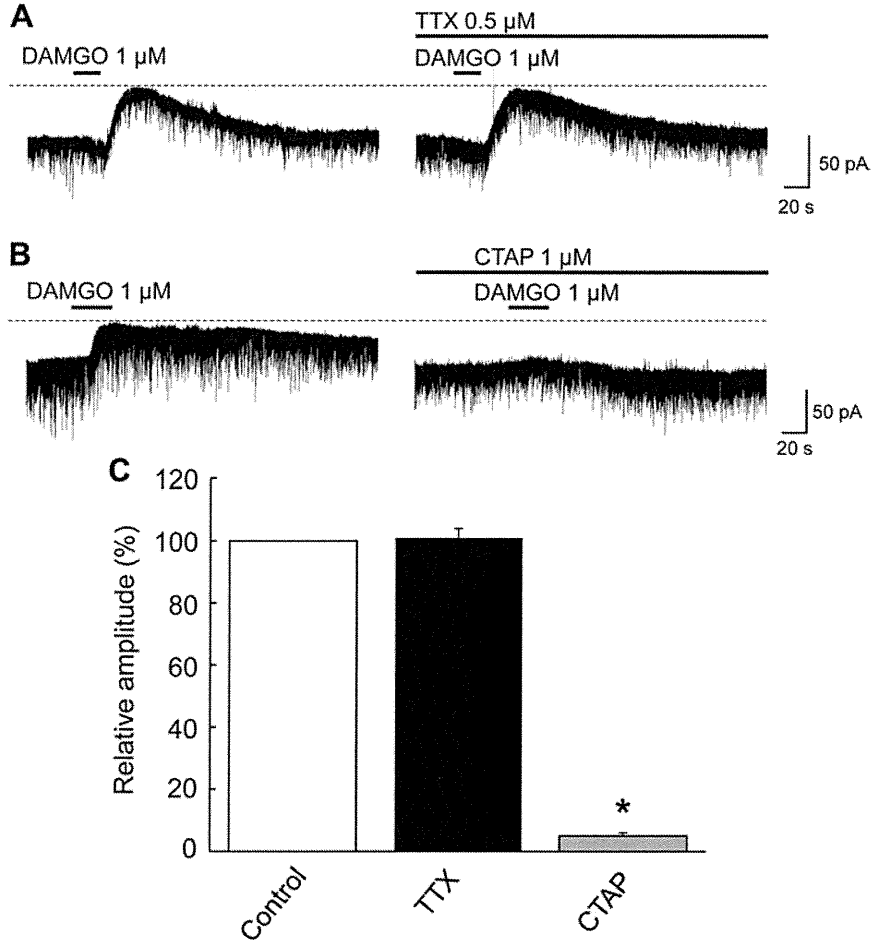

Figure 3. Effects of tetrodotoxin (TTX) or a selective μ opioid receptor (MOR) antagonist on current responses produced by [D-Ala^2-*N*-Me-Phe^4, Gly^5-ol]enkephalin (DAMGO). A, The DAMGO-induced outward current (1 μM; left) was not affected by simultaneous perfusion of TTX (0.5 μM; right). B, The DAMGO-induced current (1 μM; left) was suppressed by simultaneous application of the MOR antagonist D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2 (CTAP) (1 μM; right). C, Amplitude of DAMGO-induced current in the presence of either TTX or CTAP, relative to that in the control. Vertical bar shows SEM. *$P < 0.01$ versus control. Holding potential = −70 mV.

3.6 mM; $[K^+]_i$, 140 mM) of the solutions. This slight difference might reflect a liquid junction potential (9–10 mV) between the ACSF and patch-pipette solutions.[23] Furthermore, DAMGO-induced currents were suppressed by the application of the K^+ channel blocker Ba^{2+} (1 mM) (48% ± 3% of control, $n = 5$; $P < 0.01$; Fig. 5, A and D). These Ba^{2+} effects were examined in the presence of TTX (0.5 μM), because Ba^{2+} could influence presynaptic neuronal activity. Next, we used a pipette solution containing Cs^+ and TEA to inhibit the postsynaptic action of K^+ channels. The DAMGO-induced outward current was recorded immediately after establishing the whole-cell configuration. However, this current was reduced after the second application of DAMGO (10% ± 4% of baseline, $n = 4$; $P < 0.01$; Fig. 5, B and D). Similar results were obtained when GDP-β-S (1 mM) was used in the K-gluconate pipette solution (11% ± 5% of baseline, $n = 5$; $P < 0.01$; Fig. 5, C and D). These results indicate that the DAMGO-induced current was produced by the activation of K^+ channels through the activation of G proteins, thereby implicating G

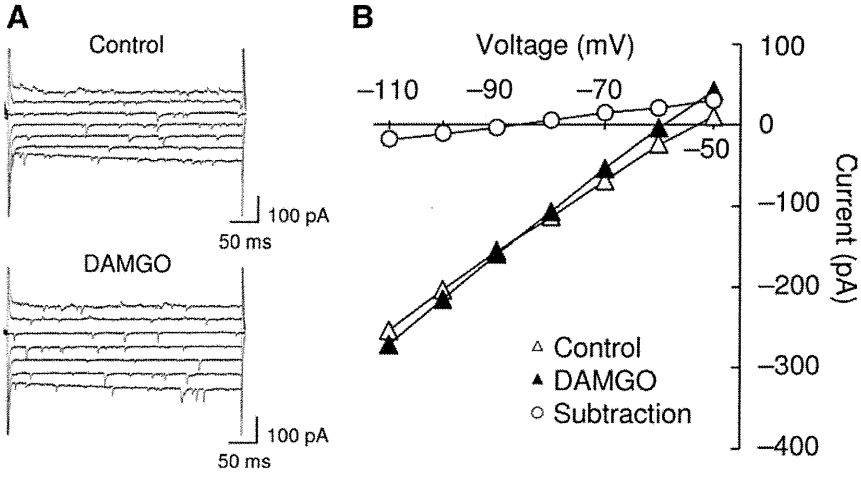

Figure 4. Voltage dependency of the [D-Ala^2-*N*-Me-Phe^4, Gly^5-ol]enkephalin (DAMGO)-induced currents. A, Voltage pulses of 400 milliseconds from the holding potential of −70 mV were given in the absence and presence of DAMGO (1 μM) current. B, Current amplitudes (measured at the end of voltage pulses) plotted against voltages in the absence (open triangles) and presence (closed triangles) of the DAMGO-induced current. The current-voltage relationship for the net DAMGO-induced current (open circles) was calculated from the difference between the current responses in the absence and presence of the DAMGO-induced current. In this case, the reversal potential of the DAMGO-induced current was approximately −90 mV.

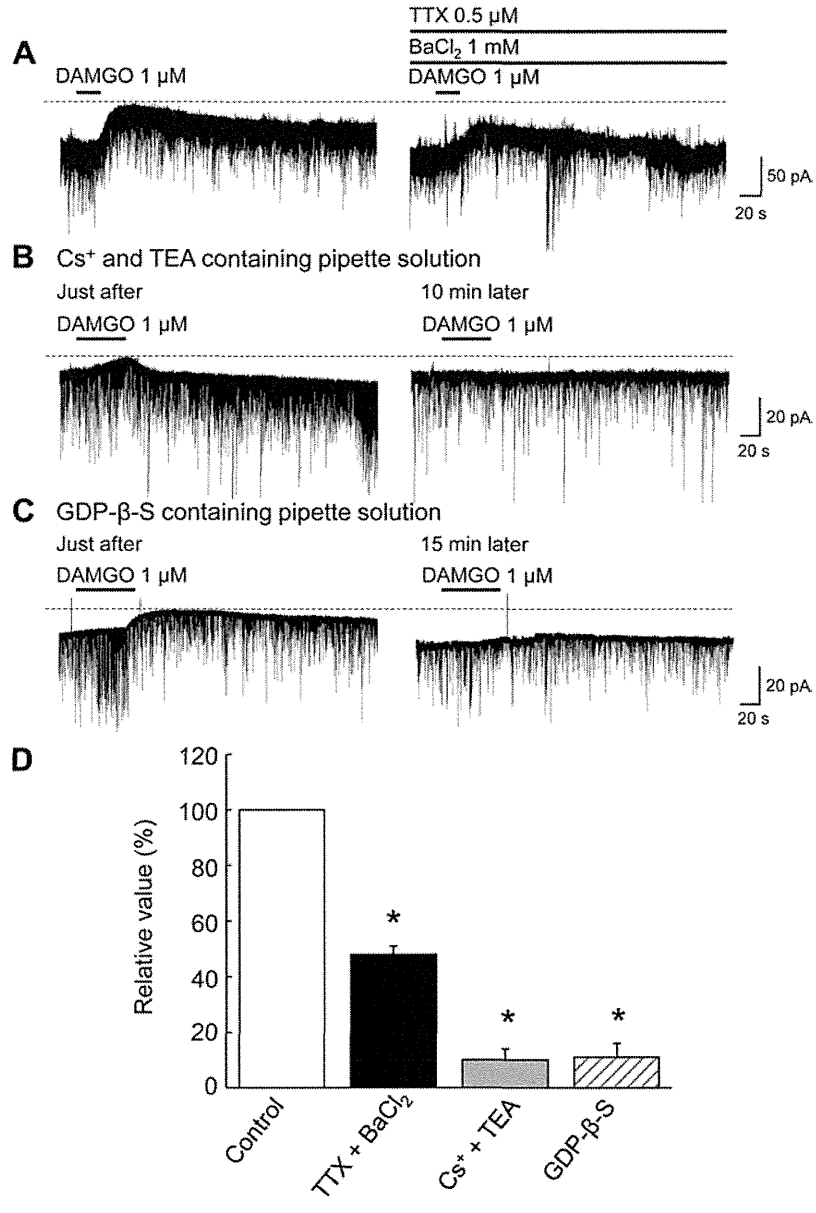

Figure 5. Effects of K^+ channel blockers and G protein–coupled receptor blocker on the [D-Ala^2-*N*-Me-Phe^4, Gly^5-ol]enkephalin (DAMGO)-induced outward current. A, The DAMGO-induced current (1 μM; left) was inhibited by simultaneous application of the K^+ channel blocker barium chloride dehydrate (Ba^{2+}) (1 mM; right; 0.5 μM tetrodotoxin [TTX] was added to remove presynaptic effects). B, The DAMGO-induced outward current was examined with Cs^+- and tetraethylammonium (TEA)-containing pipettes. DAMGO produced an outward current just after establishment of the whole-cell configuration (left), which was markedly reduced when DAMGO was again applied 10 minutes later (right). C, DAMGO induced an outward current just after establishment of whole-cell recording with a potassium gluconate pipette solution containing guanosine 5′-[β-thio]diphosphate trilithium salt (GDP-β-S) (1 mM; left). When DAMGO was again applied 15 minutes later, the current was significantly reduced (right). D, A summary of the relative amplitude of the DAMGO-induced currents under the effects of bath-applied Ba^{2+} or pipette solution containing either Cs^+ plus TEA or GDP-β-S. Vertical bar shows SEM. $*P < 0.01$ versus control. Holding potential = −70 mV.

protein–coupled inwardly rectifying potassium channels (GIRKs).

Presynaptic Actions of DAMGO in Lamina IX Neurons

In this and subsequent experiments, a cesium sulfate-based pipette solution was used to inhibit any postsynaptic effect of DAMGO through activation of K^+ channels, enabling us to focus on presynaptic actions of DAMGO. The sEPSCs had an average amplitude and frequency of 18 ± 1 pA and 23 ± 2 Hz, respectively, at a holding potential of −70 mV ($n = 9$). Superfusing DAMGO (1 μM) for 4 minutes resulted in a reversible reduction in the frequency of sEPSCs (Fig. 6A). Figure 6B shows the effects of DAMGO on the cumulative distribution of the amplitude and interevent interval of sEPSCs. DAMGO increased the proportion of sEPSCs with a significantly longer interevent interval ($P <$ 0.01) compared with the control, but it had no effect on the cumulative distribution of the amplitude of sEPSCs ($P = 0.70$). Figure 6C shows the time course of the average changes in the amplitude and frequency of sEPSCs in response to DAMGO in comparison with those of the controls ($n = 8$). A maximal reduction in sEPSC frequency was seen just after the DAMGO washout (73% ± 5% of control; $P < 0.01$; Fig. 6C), but the suppressive effect was not seen in the presence of CTAP (1 μM) (data not shown). Conversely, sEPSC amplitude was unaffected by DAMGO (95% ± 4% of control; $P =$ 0.25; Figs. 6C and 8A).

Next, we investigated the effect of DAMGO on inhibitory presynaptic input. The sIPSCs had an average amplitude and frequency of 89 ± 10 pA and 21 ± 2 Hz, respectively, at a holding potential of 0 mV ($n = 6$). DAMGO (1 μM) increased the proportion of sIPSCs with a significantly longer interevent interval ($P < 0.01$) without a

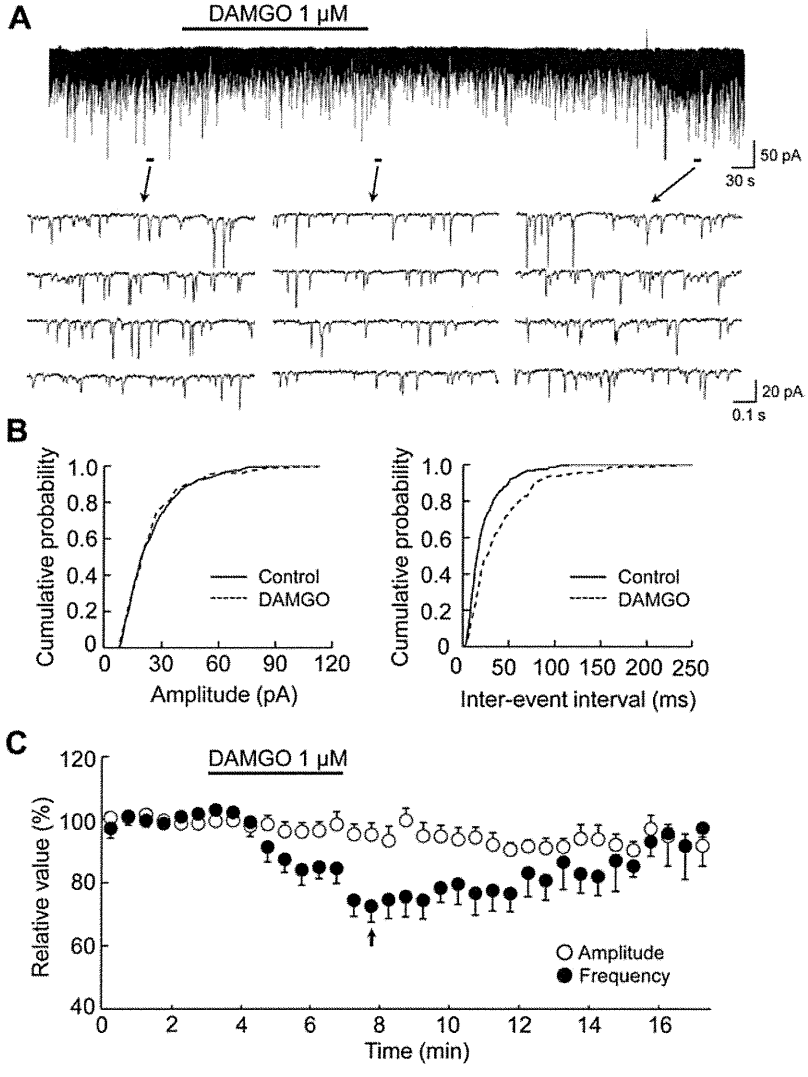

Figure 6. [D-Ala^2-*N*-Me-Phe^4, Gly^5-ol]enkephalin (DAMGO) inhibits spontaneous excitatory transmission in lamina IX neurons. A, Chart recording of spontaneous excitatory postsynaptic currents (sEPSCs) during the action of DAMGO (1 μM). In this figure and subsequent figures, 4 consecutive traces of sEPSCs for the period indicated by a short bar below the chart recording are shown in an expanded time scale. B, Cumulative distributions of the amplitude (left) and interevent interval (right) of sEPSCs before (continuous line) and during (dashed line) the action of DAMGO. DAMGO had little effect on the distribution of the amplitude (P = 0.70), but shifted the interval distribution to a longer interevent interval ($P < 0.01$), as determined by the Kolmogorov-Smirnov test. A and B were obtained from the same neuron. C, Averages of the amplitude (open circles) and frequency (closed circles) of sEPSCs under the action of DAMGO relative to control (n = 8), plotted against time. Each point with vertical bars indicates the mean value and SEM, calculated from sEPSCs measured for 30 seconds. The arrow indicates the maximal reduction in the frequency of sEPSCs. Holding potential = −70 mV.

significant change in the cumulative distribution of sIPSC amplitude (P = 0.13) (Fig. 7, A and B). A maximal reduction in sIPSC frequency was seen just after DAMGO washout (62% ± 6% of control, n = 6; $P < 0.01$; Fig. 7C), but the suppressive effect was not seen in the presence of CTAP (1 μM) (data not shown). In contrast, sIPSC amplitude was unaffected by DAMGO (102% ± 8% of control; P = 0.86; Figs. 7C and 8B).

To determine the site of the inhibitory action of DAMGO, the effects of DAMGO were examined in the presence of TTX (0.5 μM). Conduction of action potentials is blocked by TTX, so that the effects of DAMGO on the presynaptic terminal can be isolated. In the presence of TTX, postsynaptic responses to spontaneously released transmitter can be detected as miniature postsynaptic currents. TTX alone had no effect on the amplitude (98% ± 2% of control, n = 7; P = 0.34) or the frequency (101% ± 8% of control, n = 7; P = 0.94) of sEPSCs (Fig. 8A). In contrast, TTX decreased both the amplitude (70% ± 5% of control, n = 6; $P < 0.01$) and the frequency (30% ± 6% of control, n = 6; $P < 0.01$) of sIPSCs (Fig. 8B). As with the effect on spontaneous postsynaptic currents, DAMGO significantly decreased the frequency of the miniature excitatory postsynaptic currents (mEPSCs) (67% ± 5% of the TTX-treated value, n = 7; $P < 0.01$) and the miniature inhibitory postsynaptic currents (mIPSCs) (61% ± 7% of the TTX-treated value, n = 5; $P < 0.01$), but it had no effect on the amplitude of mEPSCs (P = 0.51) and mIPSCs (P = 0.33) compared with the baseline values recorded in the presence of TTX alone (Fig. 8, A and B).

DISCUSSION

MOR agonists hyperpolarize the membranes of central nervous system neurons via the activation of K^+ channels caused by MOR activation.[5,24] However, patch-clamp recordings examining μ opioid modulation of lumbar spinal motoneurons have not been reported and little is known of the effects of MOR activation in motoneurons. In this study, we examined the cellular effects of DAMGO in spinal lamina IX neurons of rat lumbar spinal cord. We demonstrated for the first time that MOR agonists produce an outward current and reduce both the excitatory and inhibitory neurotransmitter release in lamina IX neurons.

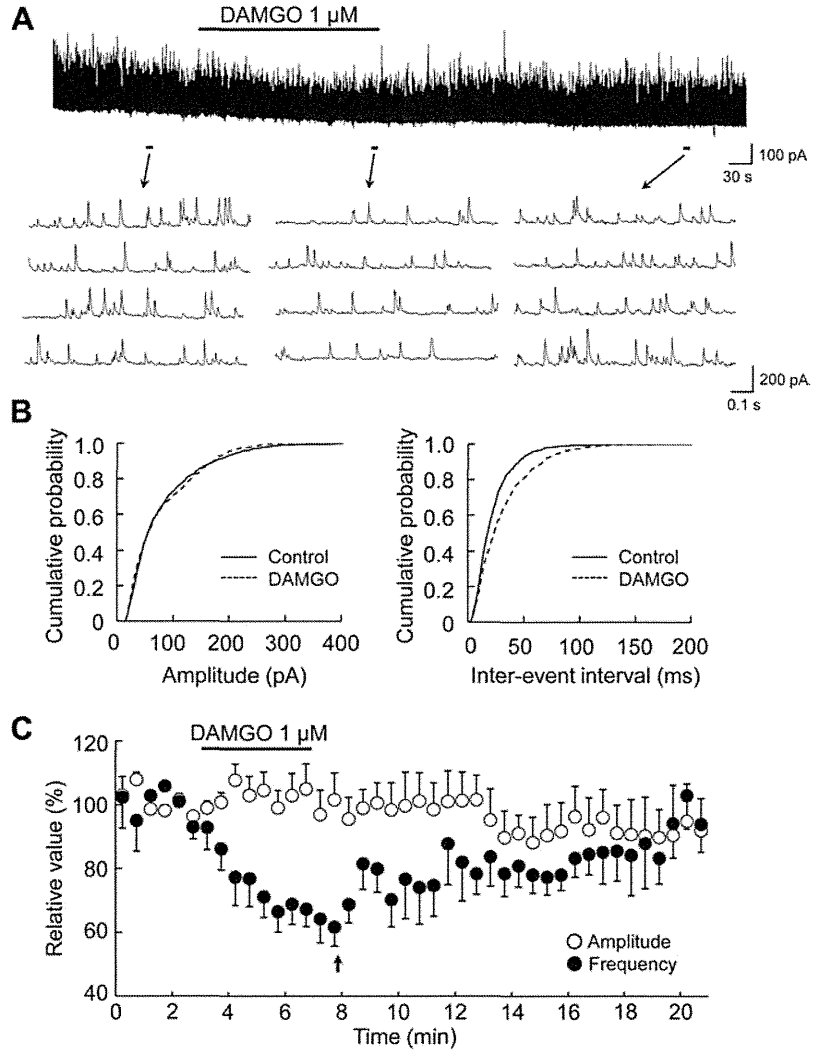

Figure 7. [D-Ala^2-*N*-Me-Phe^4, Gly^5-ol]enkephalin (DAMGO) inhibits spontaneous inhibitory synaptic transmission in lamina IX neurons. A, Chart recording of spontaneous inhibitory postsynaptic currents (sIPSCs) during the action of DAMGO (1 μM). B, Cumulative distributions of the amplitude (left) and interevent interval (right) of sIPSCs before (continuous line) and during (dashed line) the action of DAMGO. DAMGO had no effect on the distribution of the amplitude ($P = 0.13$), but shifted the interval distribution to a longer interevent interval ($P < 0.01$), as determined by the Kolmogorov-Smirnov test. A and B were obtained from the same neuron. C, Averages of the amplitude (open circles) and frequency (closed circles) of sIPSCs under the action of DAMGO relative to control ($n = 6$), plotted against time. Each point with vertical bars indicates the mean value and SEM, calculated from sIPSCs measured for 30 seconds. The arrow indicates the maximal reduction in the frequency of sIPSCs. Holding potential = 0 mV.

Bath application of DAMGO produced an outward current at −70 mV in 56% of spinal lamina IX neurons recorded. This percentage is similar to that seen in patch-clamp studies of spinal lamina II neurons.[5] The EC_{50} value of 0.10 μM for activation of MORs in postsynaptic membranes of spinal lamina IX neurons is close to that of previous reports.[25–27]

Because the DAMGO-induced current was not affected by TTX, the production of the DAMGO-induced currents is a postsynaptic effect on lamina IX neurons. Moreover, the DAMGO-induced currents were suppressed by the simultaneous application of CTAP, indicating that MORs are expressed in the postsynaptic membranes of lamina IX neurons and that their activation directly hyperpolarizes the lamina IX neurons. The polarity of the current was reversed at a potential close to the equilibrium potential of K^+ and was inhibited by perfusion with Ba^{2+} or a pipette solution containing Cs^+ and TEA, indicating the involvement of K^+ channels. The effect of Ba^{2+} was identical to that reported for currents produced by MOR activation in other central nervous system neurons.[26,28] Moreover, the currents were blocked by the addition of G protein blocker, GDP-β-S, to the pipette solution. These results indicate that the DAMGO-induced current in lamina IX neurons was produced by the activation of K^+ channels through the activation of G proteins, specifically implicating GIRKs in the effect.

The principally excitatory neurotransmitter glutamate depolarizes spinal motoneurons, whereas γ-aminobutyric acid or glycine (inhibitory neurotransmitters) hyperpolarize the motoneurons. After presynaptic release, these transmitters act on postsynaptic receptors and their integrated effect determines motoneuronal excitability.[29] We show that TTX reduced both the amplitude and frequency of sIPSCs, in agreement with previous reports.[30] These results indicate that sIPSCs depend largely on TTX-sensitive Na^+ channels, and that ongoing spontaneous inhibition occurs in the spinal ventral horn.

In addition to producing an outward current via postsynaptic MOR activation, we showed that DAMGO decreased the frequency of sEPSC and sIPSC, without influencing amplitude. Furthermore, similar suppressive effects were observed when conduction of action potentials was blocked by TTX (yielding mEPSCs and mIPSCs). Because ongoing inhibition occurred in the spinal ventral horn, the DAMGO-induced changes in sEPSCs or sIPSCs

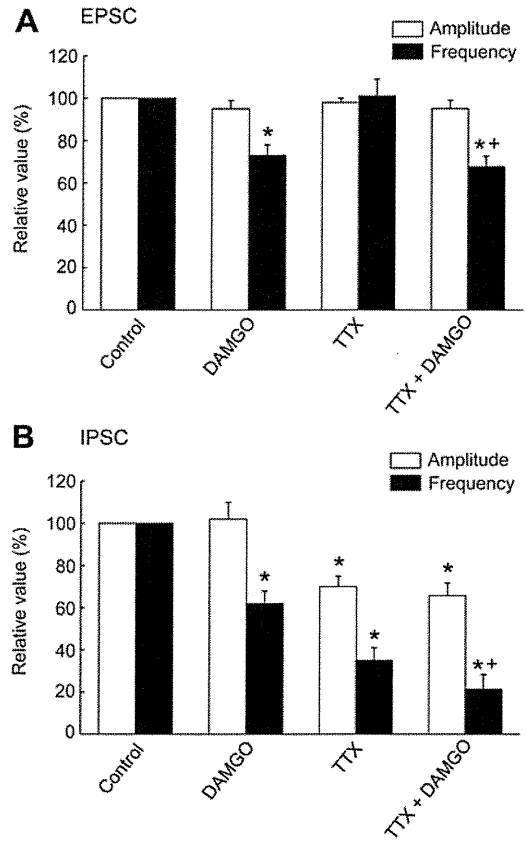

Figure 8. [D-Ala^2-*N*-Me-Phe^4, Gly^5-ol]enkephalin (DAMGO) inhibits both excitatory and inhibitory neurotransmitter release in lamina IX neurons. In A and B, summaries of the suppressive effects of DAMGO and/or tetrodotoxin (TTX) on spontaneous postsynaptic currents relative to control value are shown in bar graphs (mean and SEM). $*P < 0.01$ versus control; $+P < 0.01$ versus TTX. EPSC = excitatory postsynaptic currents; IPSC = inhibitory postsynaptic currents.

could be secondary to alterations in the activity of presynaptic inhibitory neurons. Therefore, we measured the influences of DAMGO on spontaneous postsynaptic currents with or without TTX treatment. These results suggest that MORs are located on the axon terminals of both the excitatory and inhibitory neurons and that activation of MORs decreases neurotransmitter release by both types of neurons in spinal lamina IX.

Interestingly, a suppressive effect of opioids on mIPSC frequency was not observed in adult rat spinal lamina II neurons.[4] In contrast, it has been reported that inhibitory transmission is depressed by DAMGO in substantia gelatinosa neurons of the spinal trigeminal nucleus in juvenile rat horizontal brain slices.[31] This discrepancy may reflect age-related influences or regional differences in expression of opioid system components.

Consistent with our results, MORs have been detected in spinal lamina IX of adult rats using the selective MOR probe [^{125}I]FK-33-824.[8] Moreover, MOR mRNA was detected in spinal lamina IX of rats,[7] as well as of humans,[32] using in situ hybridization. Furthermore, GIRKs have been detected in the spinal cord gray matter[33] and in brainstem motoneurons.[34] Our data indicate that the MORs of the spinal ventral horn functioned through the activation of GIRK. However, these results should be interpreted cautiously for a combination of reasons. First, the MOR activation responses in spinal lamina IX neurons may vary throughout the lifetime of an organism. MOR expression has been shown to peak at postnatal day 4 and subsequently decrease in the gray matter, except in the superficial dorsal horn, in rats.[9,35] To identify large neurons under IR-DIC microscopy in the present study, we excluded adult rats, in which spinal neurons are difficult to identify because of highly developed fibrous tissue. Second, because the spinal lamina IX neurons we studied may include propriospinal interneurons, our observations may not apply exclusively to spinal motoneurons. Further experiments that record from identified spinal motoneurons in the adult rat are required to address these caveats.

Our data indicate that MOR activation has 2 consequences in the ventral horn: (1) reduction in the release of neurotransmitters to spinal lamina IX neurons, and (2) direct hyperpolarization of spinal lamina IX neurons. Clinical significance of these opioidergic neuromodulatory effects in motor function are a matter for speculation. The MOR-mediated effects of postsynaptic hyperpolarization and the presynaptic inhibition of excitatory neurotransmitter release indicate that μ opioids can suppress excitability of spinal lamina IX neurons. This suggests that the reduction of volatile anesthetics' minimum alveolar concentration by μ opioid is attributable to direct suppression of spinal motoneuronal excitability by MOR activation. On the other hand, the presynaptic reduction of inhibitory neurotransmitter release represents the μ opioid–mediated disinhibition of the lamina IX neurons. In this respect, it is assumed that opioid-induced muscle rigidity[36] or spasticity[15,16] may be attributable to the spinal effects of μ opioids.

To assess the actual effects of MOR activation on motor pathways, several studies have analyzed transcranial motor evoked potentials in animals and humans. There are conflicting reports regarding μ opioid suppression of signaling to spinal motoneurons: some groups report suppression[11,12] whereas others do not.[37] These studies, however, used varying doses of μ opioids (fentanyl ~15 $\mu g \cdot kg^{-1}$ IV versus fentanyl 3 $\mu g \cdot kg^{-1}$ IV). Studies in spinalized rats, which restricted the sites of action of the agonists to the spinal cord, demonstrated that nociceptive responses were significantly reduced by IV-administered fentanyl (4–16 $\mu g \cdot kg^{-1}$).[38] The EC_{50} value of MORs of spinal lamina IX neurons is close to that of spinal lamina II neurons producing spinal antinociception.[26] These results suggest that μ opioids used at relevant dosages for spinal antinociception are also able to activate MORs located at the spinal ventral horn, but high doses of MOR agonists are required to suppress signaling to spinal motoneurons. Our data provide a possible explanation for these observations. We suggest that MOR-mediated suppressive effects on inhibitory neurotransmission counteract the suppressive effects on excitatory signal transduction.

In the central nervous system, inhibitory interneurons can be suppressed by μ opioids, leading to the disinhibition of cells on which such neurons terminate.[14,39,40] It has been reported that supraspinal opioid receptors solely mediate opioid-induced muscle rigidity.[41,42] Although we also

found that DAMGO can decrease inhibitory neurotransmitter release in the ventral horn, we have no direct evidence that DAMGO has excitatory effects on lamina IX neurons. In contrast, several studies have suggested that μ opioids suppressed inhibitory interneurons, which led to disinhibition of spinal motoneurons after transient spinal cord ischemia.[15,16] Because transient ischemic insult possibly altered the response to MOR agonists,[43] the increased sensitivity of the inhibitory interneurons to μ opioids may occur only after ischemic insult and cause spinal motoneuronal disinhibition.

In conclusion, our data demonstrate novel neuromodulatory effects of MOR stimulation in spinal lamina IX neurons. Our observations provide a new perspective on opioids and their receptors in motor systems.

DISCLOSURES

Name: Hiroyuki Honda, MD.
Contribution: This author helped design the study, conduct the study, analyze the data, and write the manuscript.
Attestation: Hiroyuki Honda has seen the original study data, reviewed the analysis of the data, approved the final manuscript, and is the author responsible for archiving the study files.
Name: Yasuhiko Kawasaki, PhD.
Contribution: This author helped design the study, conduct the study, and analyze the data.
Attestation: Yasuhiko Kawasaki has seen the original study data and approved the final manuscript.
Name: Hiroshi Baba, MD, PhD.
Contribution: This author helped design the study and analyze the data.
Attestation: Hiroshi Baba has seen the original study data and approved the final manuscript.
Name: Tatsuro Kohno, MD, PhD.
Contribution: This author helped design the study, analyze the data, and write the manuscript.
Attestation: Tatsuro Kohno has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
This manuscript was handled by: Steven L. Shafer, MD.

REFERENCES

1. North RA. Cellular actions of opiates and cocaine. Ann N Y Acad Sci 1992;654:1–6
2. Williams JT, Christie MJ, Manzoni O. Cellular and synaptic adaptations mediating opioid dependence. Physiol Rev 2001;81:299–343
3. Ikoma M, Kohno T, Baba H. Differential presynaptic effects of opioid agonists on Adelta- and C-afferent glutamatergic transmission to the spinal dorsal horn. Anesthesiology 2007;107:807–12
4. Kohno T, Kumamoto E, Higashi H, Shimoji K, Yoshimura M. Actions of opioids on excitatory and inhibitory transmission in substantia gelatinosa of adult rat spinal cord. J Physiol 1999;518:803–13
5. Yoshimura M, North RA. Substantia gelatinosa neurones hyperpolarized in vitro by enkephalin. Nature 1983;305:529–30
6. Rexed B. The cytoarchitectonic organization of the spinal cord in the cat. J Comp Neurol 1952;96:414–95
7. Mansour A, Fox CA, Akil H, Watson SJ. Opioid-receptor mRNA expression in the rat CNS: anatomical and functional implications. Trends Neurosci 1995;18:22–9
8. Gouarderes C, Beaudet A, Zajac JM, Cros J, Quirion R. High resolution radioautographic localization of [^{125}I]FK-33-824-labelled mu opioid receptors in the spinal cord of normal and deafferented rats. Neuroscience 1991;43:197–209
9. Kar S, Quirion R. Neuropeptide receptors in developing and adult rat spinal cord: an in vitro quantitative autoradiography study of calcitonin gene-related peptide, neurokinins, mu-opioid, galanin, somatostatin, neurotensin and vasoactive intestinal polypeptide receptors. J Comp Neurol 1995;354:253–81
10. Fernandez-Galinski SM, Monells J, Espadaler JM, Pol O, Puig MM. Effects of subarachnoid lidocaine, meperidine and fentanyl on somatosensory and motor evoked responses in awake humans. Acta Anaesthesiol Scand 1996;40:39–46
11. Scheufler KM, Zentner J. Total intravenous anesthesia for intraoperative monitoring of the motor pathways: an integral view combining clinical and experimental data. J Neurosurg 2002;96:571–9
12. Thees C, Scheufler KM, Nadstawek J, Pechstein U, Hanisch M, Juntke R, Zentner J, Hoeft A. Influence of fentanyl, alfentanil, and sufentanil on motor evoked potentials. J Neurosurg Anesthesiol 1999;11:112–8
13. Piros ET, Hales TG, Evans CJ. Functional analysis of cloned opioid receptors in transfected cell lines. Neurochem Res 1996;21:1277–85
14. Zieglgansberger W, French ED, Siggins GR, Bloom FE. Opioid peptides may excite hippocampal pyramidal neurons by inhibiting adjacent inhibitory interneurons. Science 1979;205:415–7
15. Kakinohana M, Marsala M, Carter C, Davison JK, Yaksh TL. Neuraxial morphine may trigger transient motor dysfunction after a noninjurious interval of spinal cord ischemia: a clinical and experimental study. Anesthesiology 2003;98:862–70
16. Kakinohana M, Nakamura S, Fuchigami T, Davison KJ, Marsala M, Sugahara K. Mu and delta, but not kappa, opioid agonists induce spastic paraparesis after a short period of spinal cord ischaemia in rats. Br J Anaesth 2006;96:88–94
17. Faden AI, Jacobs TP, Smith MT, Zivin JA. Naloxone in experimental spinal cord ischemia: dose-response studies. Eur J Pharmacol 1984;103:115–20
18. Rothman S. Synaptic release of excitatory amino acid neurotransmitter mediates anoxic neuronal death. J Neurosci 1984;4:1884–91
19. Honda H, Baba H, Kohno T. Electrophysiological analysis of vulnerability to experimental ischemia in neonatal rat spinal ventral horn neurons. Neurosci Lett 2011;494:161–4
20. Wang MY, Kendig JJ. Patch clamp studies of motor neurons in spinal cord slices: a tool for high-resolution analysis of drug actions. Acta Pharmacol Sin 2000;21:507–15
21. Cheng G, Kendig JJ. Enflurane decreases glutamate neurotransmission to spinal cord motor neurons by both pre- and postsynaptic actions. Anesth Analg 2003;96:1354–9
22. Wang MY, Rampil IJ, Kendig JJ. Ethanol directly depresses AMPA and NMDA glutamate currents in spinal cord motor neurons independent of actions on GABAA or glycine receptors. J Pharmacol Exp Ther 1999;290:362–7
23. Yajiri Y, Yoshimura M, Okamoto M, Takahashi H, Higashi H. A novel slow excitatory postsynaptic current in substantia gelatinosa neurons of the rat spinal cord in vitro. Neuroscience 1997;76:673–88
24. Williams JT, Egan TM, North RA. Enkephalin opens potassium channels on mammalian central neurones. Nature 1982;299:74–7
25. Christie MJ, North RA. Agonists at mu-opioid, M_2-muscarinic and $GABA_B$-receptors increase the same potassium conductance in rat lateral parabrachial neurones. Br J Pharmacol 1988;95:896–902
26. Fujita T, Kumamoto E. Inhibition by endomorphin-1 and endomorphin-2 of excitatory transmission in adult rat substantia gelatinosa neurons. Neuroscience 2006;139:1095–105
27. North RA, Williams JT, Surprenant A, Christie MJ. Mu and delta receptors belong to a family of receptors that are coupled to potassium channels. Proc Natl Acad Sci U S A 1987;84:5487–91
28. North RA, Williams JT. On the potassium conductance increased by opioids in rat locus coeruleus neurones. J Physiol 1985;364:265–80
29. Rekling JC, Funk GD, Bayliss DA, Dong XW, Feldman JL. Synaptic control of motoneuronal excitability. Physiol Rev 2000;80:767–852

30. Cheng G, Kendig JJ. Pre- and postsynaptic volatile anaesthetic actions on glycinergic transmission to spinal cord motor neurons. Br J Pharmacol 2002;136:673–84
31. Grudt TJ, Henderson G. Glycine and $GABA_A$ receptor-mediated synaptic transmission in rat substantia gelatinosa: inhibition by mu-opioid and $GABA_B$ agonists. J Physiol 1998;507:473–83
32. Peckys D, Landwehrmeyer GB. Expression of mu, kappa, and delta opioid receptor messenger RNA in the human CNS: a ^{33}P in situ hybridization study. Neuroscience 1999;88:1093–135
33. Marker CL, Stoffel M, Wickman K. Spinal G-protein-gated K^+ channels formed by GIRK1 and GIRK2 subunits modulate thermal nociception and contribute to morphine analgesia. J Neurosci 2004;24:2806–12
34. Karschin C, Dissmann E, Stuhmer W, Karschin A. IRK(1–3) and GIRK(1–4) inwardly rectifying K^+ channel mRNAs are differentially expressed in the adult rat brain. J Neurosci 1996;16:3559–70
35. Rahman W, Dashwood MR, Fitzgerald M, Aynsley-Green A, Dickenson AH. Postnatal development of multiple opioid receptors in the spinal cord and development of spinal morphine analgesia. Brain Res Dev Brain Res 1998;108:239–54
36. Benthuysen JL, Smith NT, Sanford TJ, Head N, Dec-Silver H. Physiology of alfentanil-induced rigidity. Anesthesiology 1986;64:440–6
37. Kalkman CJ, Drummond JC, Ribberink AA, Patel PM, Sano T, Bickford RG. Effects of propofol, etomidate, midazolam, and fentanyl on motor evoked responses to transcranial electrical or magnetic stimulation in humans. Anesthesiology 1992;76:502–9
38. Dong XW, Parsons CG, Headley PM. Effects of intravenous mu and kappa opioid receptor agonists on sensory responses of convergent neurones in the dorsal horn of spinalized rats. Br J Pharmacol 1991;103:1230–6
39. Johnson SW, North RA. Opioids excite dopamine neurons by hyperpolarization of local interneurons. J Neurosci 1992;12:483–8
40. Neumaier JF, Mailheau S, Chavkin C. Opioid receptor-mediated responses in the dentate gyrus and CA1 region of the rat hippocampus. J Pharmacol Exp Ther 1988;244:564–70
41. Seeber U, Kuschinsky K, Sontag KH. Inhibition by opiate narcotics of rat flexor alpha-motoneurones. Naunyn Schmiedebergs Arch Pharmacol 1978;301:181–5
42. Vankova ME, Weinger MB, Chen DY, Bronson JB, Motis V, Koob GF. Role of central mu, delta-1, and kappa-1 opioid receptors in opioid-induced muscle rigidity in the rat. Anesthesiology 1996;85:574–83
43. Formisano L, Noh KM, Miyawaki T, Mashiko T, Bennett MV, Zukin RS. Ischemic insults promote epigenetic reprogramming of mu opioid receptor expression in hippocampal neurons. Proc Natl Acad Sci U S A 2007;104:4170–5

VI. 参考資料

班会議・合同班会議

H24年度　慢性の痛み対策研究事業
難治性疼痛の実態の解明と対応策の開発に関する研究班　第１回班会議

【プログラム】

日時：平成２４年８月４日（土）１０:００～１２:４５
場所：コンベンションルームAP品川　１０Ｆ　会議室　Ｅ

10:00　今年度の班研究の目標と方向性

10:10　**疫学研究１**　【発表５分+討論５分】
（小林、中村、牛田、全員）

10:30　**疫学研究２**　【発表５分+討論５分】
難治性運動器痛（牛田、山下、平田、大森、神谷、川真田、池内）
・椎弓切除後疼痛症候群　（神谷）
・人工関節置換術後遺残性痛　（大森、池内、内田）
・手術後痛　（川真田）
11:00　神経障害に伴う痛み（安田、柴田、井関、平川）
・三叉神経痛調査　（平川）
・糖尿病性神経障害に伴う痛み　（柴田）
11:20　精神心理的要素の関与が大きな痛み　（細井）
11:30　脳外科領域のおける痛み　（片山：代理大島）

11:40　**トランスレーショナル研究１**　【発表５分+討論５分】
携帯端末を用いた総合運動支援システムの開発に関する研究　（西尾、上田）
11:50　慢性疼痛患者における大脳皮質の可塑的変化の評価法の意義に関する研究（柿木）
12:00　脳磁気刺激に関する研究　（齋藤）

12:10　**トランスレーショナル研究２**　【発表４分+討論２分】
慢性疼痛時における脊髄痛覚ニューロンの動態解明（中塚）
有痛性瘢痕研究　（牛田）

12:25　総合討論（連携方法を中心として）　司会：田倉、柴田、牛田
・医療経済、地域調査に盛り込む内容について（長久手市、琴平町）
・成果の出版
・NPOいたみ医学研究情報センターについて

（敬称略）

厚生労働科学研究費補助金

慢性の痛み対策研究事業研究班
合同班会議

◆筋骨格系慢性疼痛の疫学および病態に関する包括的研究班
◆難治性疼痛の実態の解明と対応策の開発に関する研究班
◆難治性神経因性疼痛の基礎疾患の解明と診断・治療精度を向上させるための研究班
◆情動的側面に着目した慢性疼痛の病態解明と診断・評価法の開発研究班
◆慢性疼痛の多面的評価システムの開発と客観的評価法の確立に対する研究班
◆「痛み」に関する教育と情報提供システムの構築に関する研究班

《プログラム・抄録》

合同班会議

日時：平成25年1月19日（土）9:30～14：15
会場：コンベンションルームＡＰ品川　10階ＡＢＣ会議室

痛みセンター連絡協議会

日時：平成25年1月19日（土）14:30～16：30
会場：コンベンションルームＡＰ品川　10階ＢＣ会議室

-プログラム-

9：15 ～　受付

9：30 ～　挨拶　厚生労働省疾病対策課　課長補佐　中尾　武史

【報告発表】

9：40 ～　筋骨格系慢性疼痛の疫学および病態に関する包括的研究（戸山班）

報告１　慶応義塾大学整形外科　中村　雅也

報告２　東京大学医学部附属病院医療機器管理部　住谷　昌彦

報告３　慶應義塾大学麻酔科　大西　幸

10：10～　難治性疼痛の実態の解明と対応策の開発に関する研究（牛田班）

報告１　愛知医科大学学際的痛みセンター　牛田　享宏

報告２　金沢大学医学部公衆衛生学　中村　裕之

報告３　名古屋大学医学部整形外科　平田　仁

報告４　新潟大学研究推進機構超域学術院　大森　豪

報告５　順天堂大学麻酔科学ペインクリニック講座　井関　雅子

報告６　大阪大学大学院疼痛医学寄附講座　柴田　政彦

報告７　大阪大学大学院医療経済産業政策学寄附講座　田倉　智之

11：00～　休憩

11：05～　難治性神経因性疼痛の基礎疾患の解明と診断・治療精度を向上させるための研究（池田班）

報告　信州大学医学部脳神経内科　リウマチ･膠原病内科　池田　修一

11：45～　情動的側面に着目した慢性疼痛の病態解明と診断・評価法の開発研究（南班）

報告　北海道大学大学院薬学研究院　南　雅文

12：25～　昼食

12：55～　慢性疼痛の多面的評価システムの開発と客観的評価法の確立に対する研究

報告　福島県立医科大学整形外科学講座　関口　美穂　（紺野班）

13：35～　「痛み」に関する教育と情報提供システムの構築に関する研究班　（柴田班）

報告　大阪大学大学院疼痛医学寄附講座　柴田　政彦

14：15　終了の挨拶

14：30～　痛みセンター連絡協議会　（敬称略）

-抄録-

筋骨格系慢性疼痛の疫学および病態に関する包括的研究

研究代表者：戸山芳昭
研究分担者：中村雅也、西脇祐司、住谷昌彦、大西幸、岡田泰彦

1. 筋骨格系の慢性疼痛に関わる調査研究(中村雅也、西脇祐司)

日本の人口構成にあわせて全国から無作為に抽出した 11507 人に対するアンケート調査を行い、筋骨格系の慢性疼痛の有症率は 15.4%で、男性より女性に有意に多かった。有症率は30～50 歳代が他の年齢層より高かった。疼痛部位は、腰、頚、肩、膝とその周囲が高頻度にみられた。有症者の 42%が治療をうけており、その内訳は医療機関が 19%、民間療法が 20%、その両方が 3%で、治療期間は 1 年以上が 70%と長期化していた。症状の改善は 69%に得られたが、残る 3 割は不変・悪化しており、治療に対する満足度は低かった。有症者では失業・退学、休職・休学、転職の割合(男女)が高く、また基本 ADL が障害され(男性)、IADL スコアが低かった(女性)。SF-36 の各スコアを慢性疼痛の有無で比較すると、男女ともすべてのスコアで有症者が統計学的に有意に低かった。

平成 22 年度実施の疫学調査参加者 11507 名のうち、平成 22 年度時点で慢性疼痛のあった者 1717 名、慢性疼痛のなかった者 6283 名に再度質問票を郵送し調査した。筋骨格系慢性疼痛筋骨格系の慢性疼痛の新規発生率は 11.1%であり、女性であること、職業(専門職、管理職、事務・技術職、労務・技能職)、BMI25 以上、現在飲酒者、現在喫煙者、専門学校以上の最終学歴が関連する因子であった。一方、慢性疼痛の継続は 45.2%の者にみられ、痛みの程度が強く、いつも痛い者、すでに痛みが 5 年以上継続している者、腰痛を訴える者が 1 年後に慢性疼痛が継続するハイリスク集団と考えられた。慢性疼痛の消失により心理面の QOL にも改善が示唆された

平成 22 年度及び 23 年度に実施した調査において、慢性疼痛が 2 年間継続していた協力者(550 名を想定)に対し、郵送により治療の実態、ドクターショッピングの状況、医療費等を深堀調査する。平成 24 年 12 月 8 日現在、郵送調査実施中である。年内にデータを収集し、現在データの解析中である。当日解析結果を供覧する。

2. 肥満と慢性疼痛、慢性疼痛患者の介護負担(住谷昌彦)

2-A 肥満は腰痛や頚部痛など筋骨格系疼痛の危険因子であるだけでなく、術後痛や偏頭痛など体重(機械的)負荷とも無関係な疼痛の増悪因子でもある。そこで、機械的負荷とは無関係な神経障害性疼痛患者 75 名を、body mass index (BMI)22 以上の高体重傾向群と BMI22 未満の低体重傾向群の 2 群に分け、各種痛みの parameter を評価した。その結果、痛みの NRS, MPQ-SF 総得点、MPQ-SF 感覚尺度、神経障害性疼痛重症度スコア(NPSI)は高体重群で有意に高く、NPSI の下位尺度ではアロディニア、異常感覚・知覚障害でも高体重群が有意に高かった。QOL 尺度である SF-36 は身体機能および精神的健康度、社会的 QOL のいずれも有意差はなかった。さらに、がん性疼痛およびがん開腹術後痛患者を対象に肥満(メタボリック症候群)関連遺伝子多型と疼痛強度の関連を調査し、レジスチン、アディポネクチン受容体 1、レプチン受容体等が候補 SNPs として関連を示し、今後のテーラーメイド治療の標的となりうる。

2-B 慢性疼痛患者の介護者の負担を、慢性疼痛患者の通院時に同行した介護者 90 名を対象に Zarit 介護負担尺度を用いて調査し、患者の生物心理社会的要因が介護者の負担感

に与える影響を多変量線形回帰分析を用いて評価した。慢性疼痛患者の介護負担は、透析患者や認知症患者の負担よりも高く、脊髄損傷や脳卒中のような重篤な運動麻痺を伴う患者の介護負担に準じた負担であった。患者と介護者の関係性(配偶者)、疼痛罹病期間、疼痛の病態(神経障害性疼痛)、患者の年齢、患者の性別、介護者の性別の7要因が抽出され、患者の痛みに関する破局的思考や同居の有無、疼痛に対する手術加療の有無は関連が無かった。介護者の負担尺度から介護者の抑うつ傾向も認められ、慢性疼痛患者の介護者への医療サービスの提供の必要性を検討しなければいけない。

3. 髄内腫瘍術後慢性痛の、周術期危険因子 (大西　幸)

脊髄髄内腫瘍では、術後に厳しいを生じることが少なくなく、患者のQOLにも大きく影響していることが、当院整形外科での retrospective study で明らかとなった。このアンケート調査に回答の得られた87例のうち、小児2例を除く85名を対象として、さらに麻酔記録、カルテを調査し、重回帰分析を用いて危険因子を分析し、脊髄障害性疼痛発症の手がかりを得ようとした。

85例全例を対象とすると、術前の痛み、腫瘍高位、術中血圧低下、術後24時間以後のコルチコステロイド投与が危険因子であった。高位頚髄腫瘍症例と、それ以外で、慢性痛の強さに有意差が認められたため、高位別に解析したところ、前者では、術後ボルタレンの投与および術後24時間以後のコルチコステロイドの投与が、後者では、血圧低下および手術時間が、危険因子であった。術後痛のレベル毎にみると、at level の痛みの症例では年齢が、below level の痛みの症例では、術前のNSAIDsの使用が、at および below 両者の痛みの症例では、高位頚髄腫瘍、術後経過時間が、危険因子であった。

髄内腫瘍の術後慢性痛の発生には、腫瘍高位や術前の痛み等の、症例固有の因子以外に、術中の血圧低下、手術時間、コルチコステロイド投与等の外的要因が関与していた。NSAIDsの使用に関しては、術前、術直後の痛みの強かった症例でリスクが増加したのか、本調査では結論づけられない。ステロイドは、臨床上、非常に重要な役割を果たしており、本調査の結果は慎重に扱う必要があるが、投与に際しては、注意を要する。また、脊髄腫瘍手術症例では、より厳密な術中血圧管理が求められるとともに、脊髄障害性疼痛発症に血圧低下が関与しているか、さらに調査が必要である。