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κ-Opioid receptors also increase potassium conductance

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ABSTRACT **Decrease of calcium conductance induced by opioid agonists has been reported by others for μ-, δ-, and κ-opioid receptors. On the other hand, only μ- and δ-opioid receptors have been reported to increase potassium conductance. Intracellular recordings were made from guinea pig substantia gelatinosa neurons in a brain slice. A subset of cells (29 of 83) were hyperpolarized by the κ-opioid receptor agonist U69593 with an EC_{50} of 23 nM. The κ-opioid receptor antagonist norbinaltorphimine (10 nM) blocked the hyperpolarization by U69593 but had no effect on the μ-opioid hyperpolarization present in these cells. Naloxone (300 nM) shifted the U69593 dose–response curve to the right, giving an estimated K_d for naloxone of 7.5 and 8.1 nM measured in two cells. The hyperpolarization caused by U69593 was mediated by a potassium conductance as determined with voltage clamp experiments. This demonstrates, depending on the cell type, that all three major opioid receptors (μ, δ, and κ) can increase potassium conductance as well as decrease calcium conductance.**

The substantia gelatinosa (SG) (lamina 2 of the spinal trigeminal nucleus and spinal cord) is the major site of termination of primary afferents excited by noxious stimuli (1, 2). The SG itself has been hypothesized to form a processing network (3, 4), and there are connections between the SG and nearby laminae (5, 6) that contain neurons projecting to rostral areas of the brain (7). Antinociception mediated by opioid agonists acting at μ- and κ-opioid receptors located in the dorsal horn and the SG specifically have been reported (8, 9). It is most likely that opioids reduce the excitation of dorsal horn neurons to noxious stimuli through a combination of pre- and postsynaptic mechanisms (8, 10, 11).

The actions of κ-opioid receptor agonists at the level of the single cell in the spinal cord are unknown. Activation of κ-opioid receptors has been shown to decrease calcium conductance in dorsal root ganglia; thus, these presynaptic receptors may contribute to the antinociceptive actions of κ-agonists in the spinal cord by inhibition of transmitter release (12, 13). In addition, at least a proportion of the κ-opioid receptors appear to be located on the terminals of primary afferents since the density of κ-opioid receptors in the dorsal horn falls after dorsal rhizotomy (14). This observation also indicates that κ-opioid receptors are located on cell bodies in the SG and may be an additional site for κ-opioid receptor-mediated actions.

Inhibition by opioids mediated by an increase in potassium conductance has been reported for μ- (15, 16) and δ- (17), but not κ-, opioid receptors. We report here that κ-opioid receptor activation can also increase potassium conductance in the SG of the spinal trigeminal nucleus.

MATERIALS AND METHODS

Horizontal brain slices (300 μm) containing the spinal trigeminal nucleus pars caudalis were made from 175- to 250-g male Hartley guinea pigs. Briefly, a block of brainstem including the caudal part of the medulla was placed in a vibrating microtome containing a 4°C oxygenated physiological saline solution. Slices (two or three) containing the SG were taken from near the dorsal surface of the medulla. A single slice was placed in a tissue bath through which flowed warmed (35°C) physiological saline at a rate of 1.5 ml/min. The physiological saline consisted of 126 mM NaCl, 2.5 mM KCl, 1.2 mM NaH_2PO_4, 1.3 mM $MgCl_2$, 2.4 mM $CaCl_2$, 11 mM glucose, and 25 mM $NaHCO_3$ and was gassed with 95% O_2/5% CO_2.

The SG is clearly visible in a horizontal slice of the spinal trigeminal nucleus pars caudalis. It forms a translucent band medial to the spinal trigeminal tract. Intracellular recordings were made from SG neurons using 50- to 100-MΩ electrodes containing 2 M KCl. Drugs were applied by switching from the control solution to one that differed from the control solution only in the presence of the added drug. For voltage clamp experiments, membrane currents were recorded with a single-electrode voltage-clamp amplifier (Axoclamp 2A; Axon Instruments, Foster City, CA) using switching frequencies between 3 and 5 kHz. Numerical data are presented as means ± SEM.

Drugs used and the sources are as follows: bestatin (Cambridge Research Biochemicals); D-(5α,7α,8β)-(+)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]benzeneacetamide (U69593; Sigma); [D-Ala^2,*N*-Me-Phe^4,Gly^5-ol]enkephalin (DAMGO; Sigma); [D-Pen^{2,5}]enkephalin (DPDPE; Sigma); dynorphin A (Sigma); [Met^5]enkephalin (ME; Sigma); naloxone (Research Biochemicals, Natick, MA); norbinaltorphimine (norBNI; RBI); DL-thiorphan (Bachem). Bestatin (20 μM) and DL-thiorphan (2 μM) were included in all ME and dynorphin A solutions to inhibit peptidase activity (18).

RESULTS

Intracellular recordings were made from guinea pig SG neurons in slices of spinal trigeminal nucleus pars caudalis. The selective κ-opioid agonist U69593 hyperpolarized 29 of 83 cells (35%) in a dose-dependent manner (Fig. 1). The maximum hyperpolarization ranged from a few to 32 mV and averaged 12.0 ± 1.3 mV. The EC_{50} for U69593 was 23 nM (Fig. 1*B*). The hyperpolarization peaked 3–5 min after U69593 application and the washout required 15–30 min. Repeated applications of U69593 (100 nM to 1 μM) resulted in reproducible hyperpolarizations with no diminishment of the maximum hyperpolarization, indicating that there was no desensitization in this concentration range.

The hyperpolarization caused by U69593 was antagonized by the selective κ-opioid antagonist norBNI (ref. 19; 10 nM; $n = 5$; Fig. 1*A*). This concentration of norBNI (10 nM) did not affect the μ-opioid hyperpolarization mediated by DAMGO present in these cells (Fig. 2). In addition, naloxone (300 nM)

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Abbreviations: SG, substantia gelatinosa; DAMGO, [D-Ala^2,*N*-Me-Phe^4,Gly^5-ol]enkephalin; DPDPE, [D-Pen^{2,5}]enkephalin; ME, [Met^5]-enkephalin; norBNI, norbinaltorphimine.

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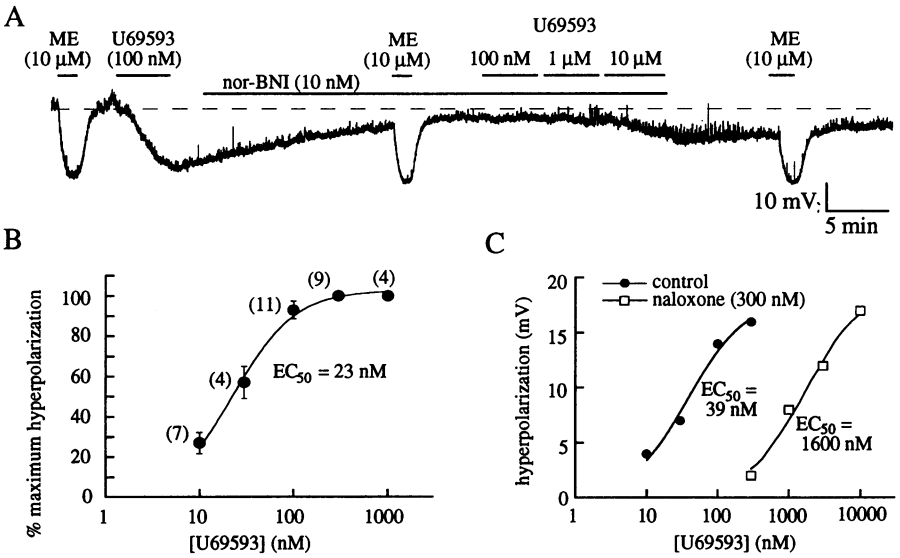

FIG. 1. κ-Opioid receptor-mediated hyperpolarization. (*A*) Recording of membrane potential from a SG neuron that was hyperpolarized by the selective κ-opioid agonist U69593. Hyperpolarization was blocked by the selective κ-opioid antagonist norBNI (10 nM). Hyperpolarization in response to ME was unchanged. Higher concentrations of U69593 overcome the antagonist block. (*B*) Dose–response to U69593. Numbers in parentheses refer to numbers of cells used to construct each data point. Error bars in this and other figures are SDs. (*C*) Naloxone shifts the U69593 dose–response curve. In this cell, the K_d for naloxone estimated from the shift is 7.5 nM.

shifted the U69593 dose–response curve to the right (Fig. 1*C*). From this single shift in the dose–response curve, the K_d for naloxone was estimated to be 7.5 and 8.1 nM in these two cells. Thus, the hyperpolarization induced by U69593 was mediated by κ-opioid receptors.

To further characterize the κ-opioid receptor-mediated effect in the SG, the populations of cells that responded to selective opioid agonists were determined. The nonselective agonist ME (3–10 μM) hyperpolarized 74 of 82 (90%) neurons. Similar results with ME have been reported in the SG of the rat spinal cord (20). In this study, the hyperpolarization was mimicked in all cases tested by the μ-opioid receptor agonist DAMGO (3 μM; n = 13) but not by the δ-opioid receptor agonist DPDPE (1 μM; n = 4). U69593 had no effect on 43 cells that were hyperpolarized by ME, while only 4 cells were hyperpolarized by U69593 but not by ME.

Since dynorphin A is an endogenous opioid thought to act at κ-opioid receptors, the effect of dynorphin A on membrane potential was examined. In contrast to the low percentage of cells (35%) hyperpolarized by U69593, dynorphin A (100 nM to 1 μM) hyperpolarized 15 of 17 SG cells (88%; Fig. 3). Seven cells were hyperpolarized by dynorphin A but not by U69593. In those cells, norBNI (10 nM) did not affect the dynorphin hyperpolarization (n = 2), while naloxone (100 nM to 1 μM; n = 4) blocked it. These results suggest that dynorphin A is acting at an opioid receptor other than the κ_1 subtype. NorBNI (100 nM) reduced the hyperpolarization caused by dynorphin A (1 μM) by 47.4% ± 9.7% (n = 5). In two cells, 1 μM norBNI also reduced the hyperpolarization caused by ME (3 μM) by 28% and 40%.

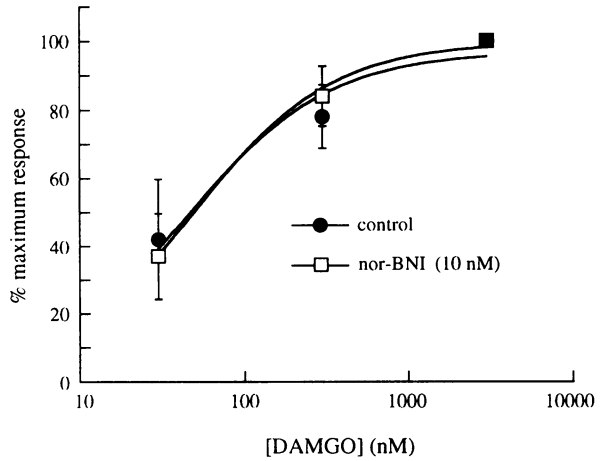

FIG. 2. NorBNI (10 nM) had no effect on hyperpolarization caused by the μ-opioid agonist DAMGO. Each data point represents the average from three cells.

The ionic mechanism of the κ-opioid receptor-mediated hyperpolarization was determined by voltage clamp experiments. Cells were held at −60 mV, voltage steps were applied to potentials ranging from −50 to −130 mV, and current/voltage curves were constructed. In 2.5 mM external potassium, U69593 caused a current that was outward at the holding potential (−60 mV) that reversed polarity at −112.2 ± 3.0 mV (n = 5; Fig. 4*A*). From the Nernst equation, the predicted potassium equilibrium potential in 2.5 mM external potassium is −107 mV, assuming an internal potassium concentration of 140 mM. In 10.5 mM potassium, the reversal potential shifted to −65.3 ± 5.8 mV (n = 3; Fig. 4*B*). The predicted potassium equilibrium potential in 10.5 mM potassium is −69 mV. These results demonstrate that the hyperpolarization caused by U69593 was mediated by an increase in potassium conductance.

DISCUSSION

Activation of κ-opioid receptors was shown to increase potassium conductance in a subset of guinea pig SG neurons. The hyperpolarization to U69593 was blocked by the selective κ-opioid antagonist norBNI at a concentration that did not affect μ-opioid hyperpolarization. The K_d of naloxone was calculated to be ≈8 nM. In a study by Chavkin *et al.* (21), naloxone was reported to have a K_d of 21–23 nM at κ-opioid receptors and 3.5–4.4 nM at μ-opioid receptors. The estimates obtained in the present study for the K_d of naloxone at the receptor mediating the U69593 hyperpolarization are lower than those obtained by Chavkin *et al.* for the κ-opioid receptor. However, using the same approach in the same preparation, we have estimated the K_d of naloxone at μ-opioid receptors to be 1.4 nM (unpublished data). Both our estimates are lower than the values found by Chavkin *et al.*,

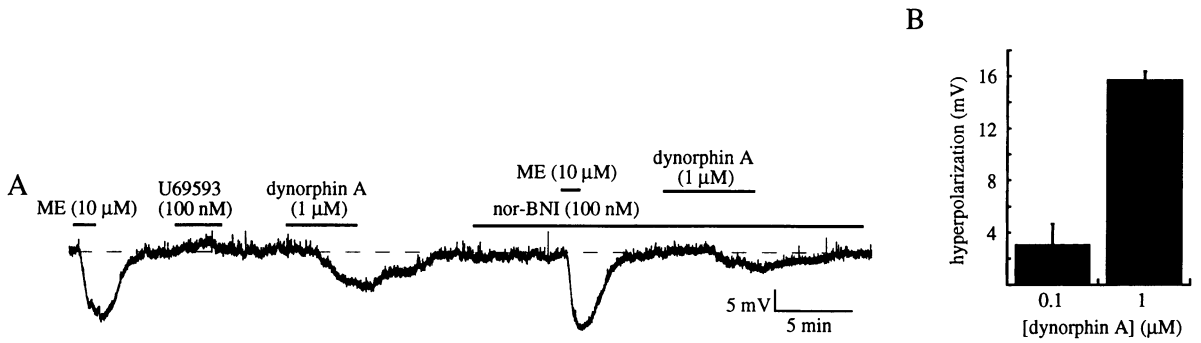

FIG. 3. Dynorphin A hyperpolarized U69593-insensitive neurons. (*A*) Dynorphin A hyperpolarized a SG neuron that was hyperpolarized by ME (10 μM) but not by U69593 (100 nM). Response to dynorphin was reduced by a high concentration of norBNI. (*B*) Amplitude of the hyperpolarizations elicited by two concentrations of dynorphin A.

but, in each study, the μ-opioid receptor has 5- to 6-fold higher affinity for naloxone than does the κ-opioid receptor.

Since U69593 and norBNI are selective for the κ_1 over the κ_2 subtype of κ-opioid receptors (22), the hyperpolarization caused by U69593 is likely to be mediated by κ_1-opioid receptors. The dynorphin A hyperpolarization in the absence of a U69593 hyperpolarization was not blocked by norBNI at the concentration that blocked the U69593 response, but it was blocked by the nonspecific opioid antagonist naloxone. One possibility is that dynorphin A was acting at μ-opioid receptors. Consistent with this, the percentage of cells hyperpolarized by dynorphin A and μ agonists is similar. Binding studies suggest that dynorphin A is not selective for the κ_1- over μ-opioid receptors (22), and it has been reported that dynorphin A can act at μ-opioid receptors (23). Alternatively, dynorphin A may act at κ_2-opioid receptors. However, μ-opioid receptors have been reported to have at least 10-fold higher affinity for dynorphin A than do κ_2 receptors (22). In addition, κ_2 receptors are absent or present at very low levels in guinea pig (24). It is likely that dynorphin A was acting at μ-opioid receptors, but it is not possible to rule out an additional action at κ_2 receptors.

An action mediated by κ-opioid receptors in SG cells was suggested from binding studies showing that the highest levels of κ-opioid receptors in the spinal cord are found in the SG (25–27). At least some of the receptors are postsynaptic since half remain after dorsal rhizotomy (14). The presence of endogenous dynorphin terminals in the superficial dorsal horn (28, 29) suggests that under the right conditions synaptically released dynorphin could increase potassium conductance.

Inhibiting the activity of SG neurons is one way in which signaling in the pain pathway could be reduced. The excitation of laminae 3–5 dorsal horn neurons by noxious stimuli was reduced by systemic and locally applied κ-opioid receptor agonists (10, 11, 30). In addition, it has been reported that a higher level of antinociception can be obtained by using μ-opioid agonists compared to κ-opioid agonists (8, 31). This could be explained by the finding that while only a portion of SG cells are hyperpolarized by κ-opioids, μ-opioids hyperpolarize nearly all SG cells. The larger percentage of cells inhibited by μ-opioids may provide for a more complete block of the relay of the nociceptive signal.

In summary, as has been reported for μ- and δ-opioid receptors, we have found that activation of κ-opioid receptors can modulate the activity of neurons by increasing potassium conductance.

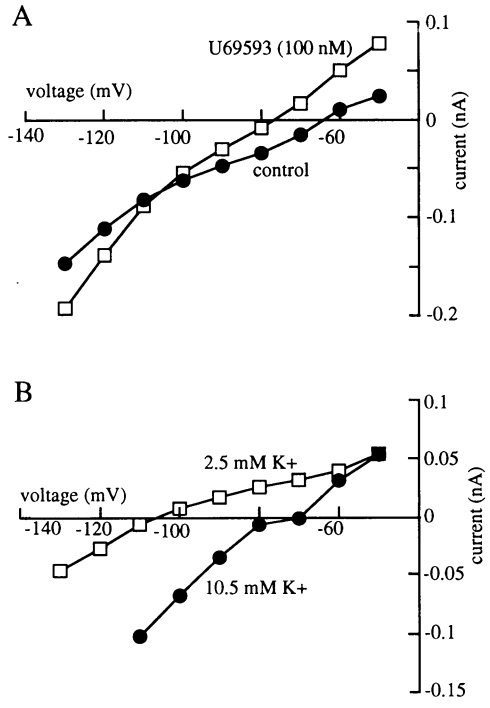

FIG. 4. κ-opioid hyperpolarization is mediated by an increase in potassium conductance. (*A*) Current/voltage curves were constructed for this cell under voltage clamp. The cell was held at −60 mV and stepped to different potentials for 500 ms. (*B*) U69593 currents obtained by subtracting the control current/voltage curve from the U69593 current/voltage curve. Increasing the external potassium concentration from 2.5 to 10.5 mM shifted the reversal potential to the right, as expected for a current mediated by a potassium conductance.

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1. Light, A. R. & Perl, E. R. (1979) *J. Comp. Neurol.* **186,** 117–132.
2. Sugiura, Y., Lee, C. L. & Perl, E. R. (1986) *Science* **234,** 358–361.
3. Gobel, S. (1978) *J. Comp. Neurol.* **180,** 395–414.
4. Bennett, G. J., Abdelmoumene, M., Hayashi, H. & Dubner, R. (1980) *J. Comp. Neurol.* **194,** 809–827.
5. Gobel, S., Falls, W. M., Bennett, G. J., Abdelmoumene, M., Hayashi, H. & Humphrey, E. (1980) *J. Comp. Neurol.* **194,** 781–807.
6. Light, A. R. & Kavookjian, A. M. (1988) *J. Comp. Neurol.* **267,** 172–189.
7. Giesler, G. J., Menetrey, D., Guilbaud, G. & Besson, J.-M. (1976) *Brain Res.* **118,** 320–324.
8. Millan, M. J. (1990) *Trends Pharmacol. Sci.* **11,** 70–76.
9. Herrero, J. F. & Headley, P. M. (1991) *Br. J. Pharmacol.* **104,** 166–170.

10. Fleetwood-Walker, S. M., Hope, P. J., Mitchell, R., El-Yassir, N. & Molony, V. (1988) *Brain Res.* **451,** 213–226.
11. Dong, X.-W., Parsons, C. G. & Headley, P. M. (1991) *Br. J. Pharmacol.* **103,** 1230–1236.
12. Macdonald, R. L. & Werz, M. A. (1986) *J. Physiol. (London)* **377,** 237–249.
13. Gross, R. A. & Macdonald, R. L. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 5469–5473.
14. Besse, D., Lombard, M. C., Zajac, J. M., Roques, B. P. & Besson, J. M. (1990) *Brain Res.* **521,** 15–22.
15. Williams, J. T. & North, R. A. (1984) *Mol. Pharmacol.* **26,** 489–497.
16. Wimpey, T. L. & Chavkin, C. (1991) *Neuron* **6,** 281–289.
17. Mihara, S. & North, R. A. (1986) *Br. J. Pharmacol.* **88,** 315–322.
18. Williams, J. T., Christie, M. J., North, R. A. & Roques, B. P. (1987) *J. Pharmacol. Exp. Ther.* **243,** 397–401.
19. Takemori, A. E., Ho, B. Y., Naeseth, J. S. & Portoghese, P. S. (1988) *J. Pharmacol. Exp. Ther.* **246,** 255–258.
20. Yoshimura, M. & North, R. A. (1983) *Nature (London)* **305,** 529–530.
21. Chavkin, C., James, I. F. & Goldstein, A. (1982) *Science* **215,** 413–415.
22. Nock, B., Giordano, A. L., Cicero, T. J. & O'Conner, L. H. (1990) *J. Pharmacol. Exp. Ther.* **254,** 412–419.
23. Chavkin, C., Henriksen, S. J., Siggins, G. R. & Bloom, F. E. (1985) *Brain Res.* **331,** 366–370.
24. Zukin, R. S., Eghbali, M., Olive, D., Unterwald, E. & Tempel, A. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 4061–4065.
25. Morris, B. J. & Herz, A. (1987) *Naunyn-Schmiedeberg Arch. Pharmacol.* **336,** 240–243.
26. Mansour, A., Khachaturian, H., Lewis, M. E., Akil, H. & Watson, S. J. (1988) *Trends Neurosci.* **11,** 308–314.
27. Sharif, N. A., Hunter, J. C., Hill, R. G. & Hughes, J. (1988) *Neurosci. Lett.* **86,** 272–278.
28. Vincent, S. R., Hokfelt, T., Christensson, I. & Terenius, L. (1982) *Neurosci. Lett.* **33,** 185–190.
29. Cruz, L. & Basbaum, A. I. (1985) *J. Comp. Neurol.* **240,** 331–348.
30. Hope, P. J., Fleetwood-Walker, S. M. & Mitchell, R. (1990) *Br. J. Pharmacol.* **101,** 477–483.
31. Millan, M. J. (1989) *J. Pharmacol. Exp. Ther.* **251,** 334–341.