The Journal of Neuroscience, August 1994, 14(8): 4965–4971

Inhibitory Actions of δ_1-, δ_2-, and μ-Opioid Receptor Agonists on Excitatory Transmission in Lamina II Neurons of Adult Rat Spinal Cord

Steven R. Glaum,[1] Richard J. Miller,[1] and Donna L. Hammond[2]

Departments of [1]Pharmacological and Physiological Sciences and [2]Anesthesia and Critical Care, The University of Chicago, Chicago, Illinois 60637

This study examined the electrophysiological consequences of selective activation of δ_1-, δ_2-, or μ-opioid receptors using whole-cell recordings made from visually identified lamina II neurons in thin transverse slices of young adult rat lumbar spinal cord. Excitatory postsynaptic currents (EPSCs) or potentials (EPSPs) were evoked electrically at the ipsilateral dorsal root entry zone after blocking inhibitory inputs with bicuculline and strychnine, and NMDA receptors with D-2-amino-5-phosphonopentanoic acid. Bath application of the μ receptor agonist [D-Ala^2, *N*-MePhe^4, Gly^5-ol]enkephalin (DAMGO) or the δ_1 receptor agonist [D-Pen^2, D-Pen^5]enkephalin (DPDPE) produced a log-linear, concentration-dependent reduction in the amplitude of the evoked EPSP/EPSC. By comparison, the δ_2 receptor agonist [D-Ala^2, Glu^4]deltorphin (DELT) was unable to reduce the evoked EPSP/EPSC by more than 50% at 100 μM, the highest concentration tested. At concentrations that reduced evoked EPSP/EPSCs by 40–60%, neither DAMGO, DPDPE, nor DELT decreased the amplitude of the postsynaptic current produced by brief pressure ejection of (*S*)-α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid, suggesting a presynaptic site of action of these opioid receptor agonists. Bath application of 200 nM naltriben (NTB), a δ_2 receptor antagonist, competitively increased the EC_{75} of DELT by 15.3-fold, but did not antagonize either DPDPE or DAMGO. The EC_{75} of DELT was further increased by 169.7-fold in the presence of 1 μM NTB. However, this high concentration of NTB also increased the EC_{50} of DPDPE by about threefold in a noncompetitive manner and antagonized DAMGO in a noncompetitive manner. In contrast, bath application of 33 or 100 nM 7-benzylidenenaltrexone (BNTX), a δ_1 receptor antagonist, produced a concentration-dependent, noncompetitive antagonism of DPDPE, but did not antagonize DELT. A modest noncompetitive antagonism of DAMGO occurred in the presence of 100 nM BNTX. Bath application of 500 nM naloxone competitively antagonized DAMGO as well as DPDPE, increasing their EC_{50} values by 13.3- and 2.5-fold, respectively. These results provide the first electrophysiological demonstration of functional subtypes of the δ-opioid receptor in rat spinal cord and indicate that activation of either δ_1- or δ_2-opioid receptors inhibits excitatory, glutamatergic afferent transmission in the spinal cord. This effect may mediate the ability of δ_1 or δ_2 receptor agonists to produce antinociception when administered intrathecally in the rat.

[Key words: DPDPE, deltorphin, spinal cord slice, EPSP, δ-opioid receptor, DAMGO, naltriben, 7-benzylidenenaltrexone (BNTX), naloxone]

The dorsal horn of the spinal cord is an important site for the production of antinociception by μ- and δ-opioid receptor agonists (Yaksh, 1993). Postsynaptic, as well as presynaptic, sites of action are thought to mediate this effect. A postsynaptic site of action is suggested by the existence of enkephalinergic synapses on dorsal horn neurons (Hunt et al., 1980; Aronin et al., 1981; Ruda, 1982; Sumal et al., 1982) and by the ability of opioid receptor agonists to inhibit the excitation of dorsal horn neurons produced by iontophoretic application of excitatory amino acids (Belcher and Ryall, 1978; Zieglgänsberger and Tulloch, 1979; Willcockson et al., 1984). In addition, μ-opioid receptor agonists hyperpolarize and decrease the input resistance of dorsal horn neurons in slices of the rat spinal cord or spinal trigeminal nucleus (Murase et al., 1982; Jeftinija, 1988; Grudt and Williams, 1994), primarily by increasing one or more K^+ conductances (Yoshimura and North, 1983; Grudt and Williams, 1994). A presynaptic site of action is suggested by the ability of opioid receptor agonists to inhibit the release of neurotransmitters from primary afferent neurons (Go and Yaksh, 1987; Collin et al., 1991; Kangrga and Randić, 1991) and by the marked decrease in the number of opioid receptor binding sites in the dorsal horn that occurs after dorsal rhizotomy or the destruction of small diameter primary afferents by capsaicin (reviewed by Besson et al., 1989). In addition, low Ca^{2+}/high Mg^{2+}–containing solutions reduce the inhibitory effects of opioid receptor agonists on synaptic transmission in the dorsal horn (Murase et al., 1982; Hori et al., 1992). Finally, opioid receptor agonists decrease both the frequency of miniature EPSPs and the amplitude of evoked EPSPs in dorsal horn neurons in slices of rat spinal cord (Hori et al., 1992).

These data provide strong support for opioid modulation of excitatory afferent synaptic transmission in the spinal cord, particularly by μ-opioid receptors. However, the role of δ-opioid receptors is less clear. The prototypic δ-opioid receptor agonist [D-Pen^2, D-Pen^5]enkephalin (DPDPE) does not affect the membrane potential or the amplitude of evoked EPSPs recorded in

Received July 7, 1993; revised Jan. 14, 1994; accepted Feb. 17, 1994.

This work was supported by United States Public Health Service Grants DA 06736 to D.L.H. and DA 02575 and DA 02121 to R.J.M.

Correspondence should be addressed to Dr. Steven R. Glaum, Department of Pharmacological and Physiological Sciences, The University of Chicago, 947 East 58th Street, Chicago IL 60637.

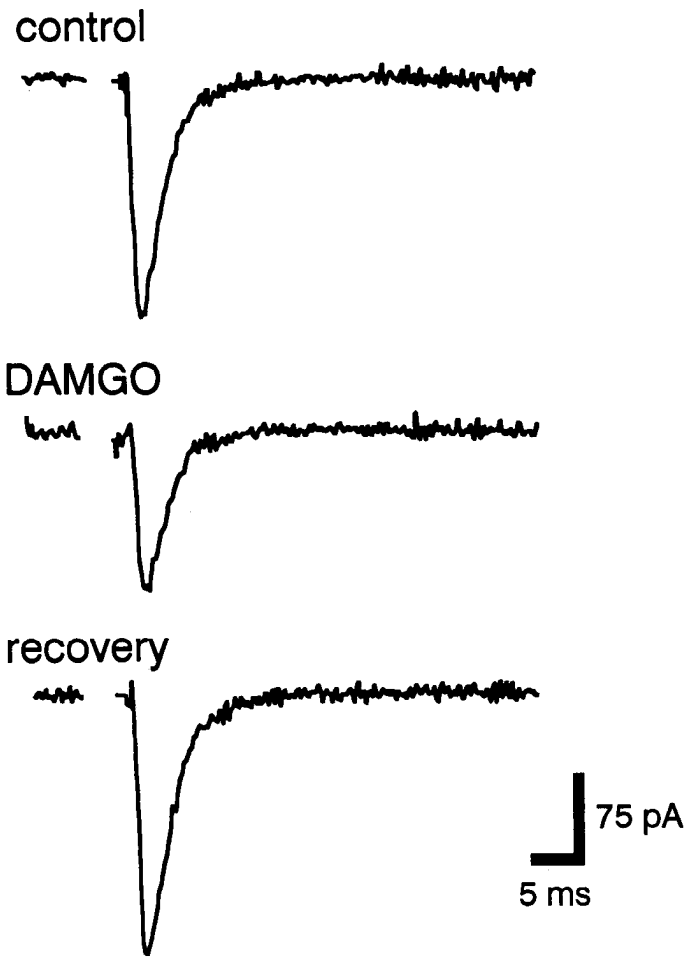

Figure 1. High-resolution trace of monosynaptic EPSCs evoked in a lamina II neuron and its inhibition by 0.3 μM DAMGO. The neuron was voltage clamped at −70 mV and EPSCs were evoked at 0.1 Hz. The averaged (n = 5) trace of the EPSC is shown immediately before application of DAMGO in the perfusate (*control*), 15 sec after DAMGO application (*DAMGO*), and following a 2 min washout (*recovery*). The stimulus artifact has been retouched in the figure.

superficial dorsal horn neurons in slices of rat spinal cord or spinal trigeminal nucleus (Jeftinija, 1988; Grudt and Williams, 1994). Similarly, [D-Pen^2, L-Pen^5]enkephalin, a close structural analog of DPDPE, does not affect the frequency of miniature EPSPs recorded in lamina I neurons in slices of rat spinal cord (Hori et al., 1992), leading the authors to conclude that excitatory synaptic transmission is predominantly modulated by μ receptors (Jeftinija, 1988; Hori et al., 1992). Yet, highly selective δ-opioid receptor agonists produce antinociception and inhibit the responses of dorsal horn neurons to noxious stimuli when administered intrathecally in the rat (Sullivan et al., 1989; Malmberg and Yaksh, 1992; Stewart and Hammond, 1993). Moreover, recent evidence, much of it obtained from *in vivo* studies, suggests the existence of at least two subtypes of the δ-opioid receptor (reviewed by Sofuoglu et al., 1991; Mattia et al., 1992; Stewart and Hammond, 1993), both of which are now implicated in the production of antinociception at the level of the spinal cord (Sofuoglu et al., 1991, 1993; Stewart and Hammond, 1993; but see Mattia et al., 1992). The present study was therefore undertaken to reexamine the role of δ-opioid receptors, and in particular the δ_1 and δ_2 subtypes of this receptor, in the modulation of excitatory afferent synaptic transmission in the spinal cord. Whole-cell recordings were made of visually identified neurons in lamina II, a principal termination site for small diameter primary afferent fibers that convey nociceptive information to the central nervous system (reviewed by Willis and Coggeshall, 1991; Light, 1992), in thin transverse slices of the spinal cord obtained from young adult rats. The ability of the δ_1 receptor agonist DPDPE (Jiang et al., 1991; Sofuoglu et al., 1991, 1993), the δ_2 receptor agonist [D-Ala^2, Glu^4]deltorphin (DELT) (Jiang et al., 1991; Sofuoglu et al., 1991; Stewart and Hammond, 1993), or the μ receptor agonist [D-Ala^2, *N*-MePhe^4, Gly^5-ol]enkephalin (DAMGO) to inhibit evoked excitatory postsynaptic potentials/currents (EPSP/EPSCs) in these neurons was determined. In addition, the pharmacologic specificity of this inhibition was characterized using the δ_1 receptor antagonist 7-benzylidenenaltrexone (BNTX) (Portoghese et al., 1992; Sofuoglu et al., 1993) and the δ_2 receptor antagonist naltriben (NTB) (Sofuoglu et al., 1991; Stewart and Hammond, 1993).

Materials and Methods

Slice preparation. Transverse slices of the rat spinal cord were prepared as previously described (Bleakman et al., 1992). Briefly, Sprague–Dawley rats (Holtzman, Madison, WI) of either sex, aged between 28 and 60 d, were deeply anesthetized with ether and a laminectomy was performed that exposed the entire thoracolumbar enlargement and an additional three to four segments rostrocaudally. The rat was then killed by ether overdose, and the spinal cord and surrounding tissue were immediately removed to a dissecting dish. The spinal cord was freed from the surrounding tissue and 175–225 μm transverse slices were prepared from the region of the thoracolumbar enlargement with a vibrating microtome.

Slices were allowed to recover for at least 1 hr in 32°C artificial cerebrospinal fluid (ACSF), which contained (in mM) NaCl, 126; $NaHCO_3$, 26.2; NaH_2PO_4, 1; KCl, 3; $MgSO_4$, 1.5; $CaCl_2$, 2.5; glucose, 10, and was continuously gassed with 95% O_2, 5% CO_2. A single slice was then transferred to the recording chamber (volume of 1 ml), where it was continuously perfused (4–6 ml min^{-1}) at room temperature with ACSF containing 50 μM D-2-amino-5-phosphonopentanoic acid, 10 μM bicuculline, and 10 μM strychnine. Under these conditions, a complete exchange of perfusate occurred in 10–25 sec. Whole-cell recordings were made with 2–5 MΩ patch electrodes containing in mM: K-gluconate, 145; $MgCl_2$, 2; $CaCl_2$, 0.1; HEPES, 5; EGTA, 1.1; K_2ATP, 5 (pH 7.2). Experiments were typically performed under voltage-clamp conditions in the discontinuous mode of the amplifier at a holding potential of −60 to −80 mV. In discontinuous current-clamp experiments, resting V_m was held constant at −60 to −80 mV by direct current injection. Monosynaptic EPSP/EPSCs were evoked by single, subthreshold stimuli (30–120 μsec, 3–10 V) delivered at 0.1–0.2 Hz by a bipolar tungsten electrode placed in the ipsilateral dorsal root entry zone. These EPSP/EPSCs are denoted generically as EPSCs in the subsequent text. Addition of 10 μM 6,7-dinitroquinoxaline-2,3-dione completely suppressed the evoked EPSCs, indicating they were mediated by the actions of glutamate on the α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA)/kainate receptor subtype (Schneider and Perl, 1988; Yoshimura and Jessell, 1990).

Experimental design. Experiments were performed on 66 visually identified lamina II neurons. A single neuron was examined per slice. Each slice was exposed to between one and six applications of DAMGO, DPDPE, or DELT alone or in combination with either NTB, BNTX, or naloxone. The opioid receptor agonists were applied in the perfusate for 15–30 sec in the absence or continuous presence of the antagonist, which was also applied in the perfusate. In all cases, slices were washed for 5–25 min between drug applications to ensure sufficient washout of opioid effects. Desensitization under these conditions was not evident as responses to reapplication of a given dose of agonist varied by <5%.

The potential interaction of DAMGO, DPDPE, or DELT with postsynaptic glutamate receptors mediating the evoked EPSCs was studied in six additional neurons. For these studies, 1 mM (*S*)-AMPA was applied by brief pressure ejection (2–9 psi, 10–50 msec; Bleakman et al., 1992) to the soma of visually identified laminae II neurons in the absence and presence of equieffective concentrations of DAMGO, DPDPE, or

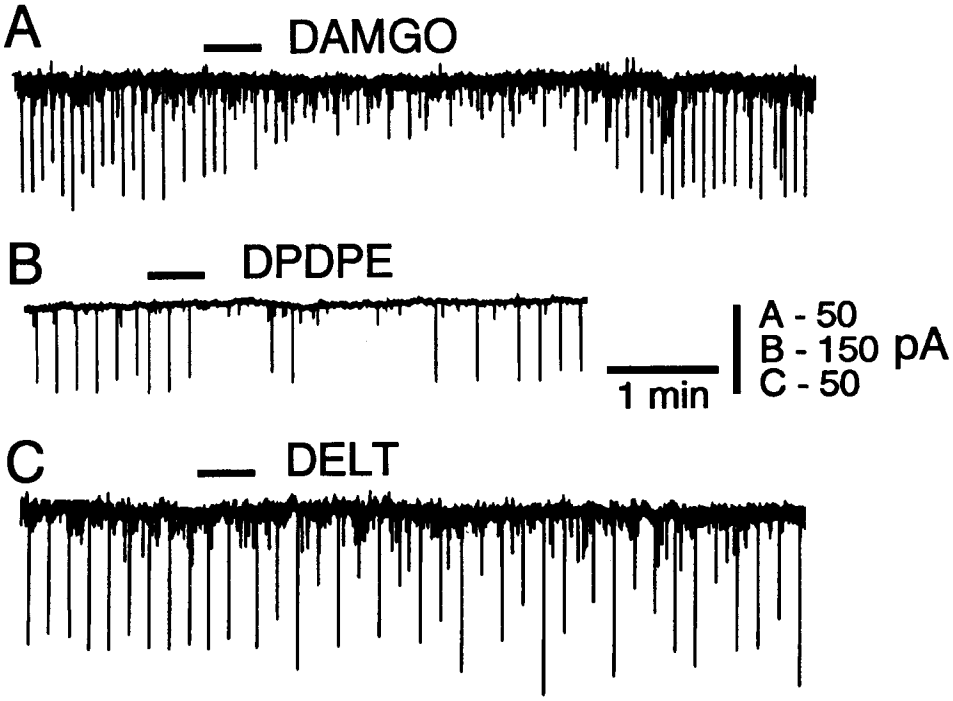

Figure 2. Representative chart recordings of evoked EPSCs (regular downward deflections) in lamina II neurons and their inhibition by, *A*, 0.3 μM DAMGO; *B*, 20 μM DPDPE; or *C*, 10 μM DELT. These concentrations of DAMGO, DPDPE, and DELT reduced the mean EPSC for all neurons to 59.1 ± 5.8% (n = 9), 38.4 ± 3.1% (n = 5), and 52.1 ± 6.4% (n = 5) of control, respectively. EPSCs were evoked at 0.2 Hz (*A*) or 0.1 Hz (*B* and *C*). The opioid agonists were applied in the perfusate for the duration indicated by the *horizontal bars*.

DELT. Neurons were examined at holding potentials between −50 and −70 mV; 0.5 μM tetrodotoxin was added to the perfusate for these studies.

Statistical analysis. Drug effects were determined by comparing the averaged peak amplitude of five consecutive EPSCs evoked immediately prior to drug application ($I_{control}$) to the averaged peak amplitude of five consecutive EPSCs evoked at the apparent peak of the response ($I_{treatment}$). $I_{treatment}$ was typically determined 20–40 sec after the onset of drug application and was expressed as percentage of control: ($I_{treatment}/I_{control}$) × 100. Concentration–effect lines for DELT, DPDPE, or DAMGO were calculated using least squares linear regression analysis of the individual data and the concentration of agonist that produced 50% inhibition was determined. In the case of DAMGO or DPDPE, both of which completely suppressed the EPSCs, this criterion corresponded to 50% of control, or the EC_{50}. In the case of DELT, which suppressed the EPSCs by only 50% at the highest concentration tested (100 μM), this criterion corresponded to 75% of control, or the EC_{75}. Fieller's theorem was used to determine 95% confidence limits (CL) of the EC_{50} or EC_{75} values (Finney, 1964). The concentration–effect lines of each agonist in the absence and presence of the antagonist were compared for parallelism as described by Tallarida and Murray (1987). Where the lines were parallel, an analysis of covariance was used to determine the statistical significance of differences in the EC_{50} or EC_{75} values of the agonist (Zar, 1974).

Drugs. DAMGO, DPDPE, DELT, naloxone, bicuculline, D-2-amino-5-phosphonopentanoic acid, tetrodotoxin, and strychnine were purchased from Sigma Chemical Co. (St. Louis, MO). NTB was synthesized by Mr. Peter Yonan of G. D. Searle and Co. (Skokie, IL). BNTX was purchased from Research Biochemicals, Inc. (Natick, MA). (*S*)-AMPA was purchased from Tocris Neuramin (Bristol, England).

Results

μ- and δ-opioids reversibly inhibit excitatory transmission

Addition of 0.003–3.0 μM DAMGO to the perfusate produced a reversible reduction in monosynaptic EPSCs (Fig. 1). The reduction in EPSCs was qualitatively graded and persisted for 2–4 min after the application of DAMGO was terminated (Fig. 2*A*). At concentrations ≤0.3 μM DAMGO, the reduction in EPSCs was not associated with a detectable change in the holding current, suggesting that it was mediated at a presynaptic locus. Moreover, repeated application of DAMGO at concentrations ≤0.3 μM produced reproducible reductions in EPSCs, indicating an absence of desensitization. Other investigators have also reported no desensitization to the presynaptic effects of μ receptor agonists in the central nervous system (North, 1993; Rhim et al., 1993). Application of a higher concentration of DAMGO (3.0 μM) produced an outward current of <25 pA in 4 of 10 cells. This current was absent or sharply reduced after a second application of 3.0 μM DAMGO. The reduction in EPSCs produced by DAMGO was concentration-dependent (Fig. 3*A*) and a complete suppression of evoked EPSCs (to 0–5% of control) occurred in 3 of 10 neurons after application of 3.0 μM DAMGO; one of these three neurons exhibited an outward current. The EC_{50} (95% CL) of DAMGO was 0.5 (0.3–1.1) μM.

Application of 0.2–100 μM DPDPE also produced a reversible reduction in EPSCs (Fig. 2*B*). The duration of the reduction in EPSCs produced by DPDPE often persisted longer (up to 20 min) than the inhibition produced by an equieffective concentration of DAMGO (2–4 min). The reduction in EPSCs was not accompanied by any appreciable changes in holding current (defined as >5 pA) even at 100 μM DPDPE, the highest concentration tested. Moreover, no shift occurred in the whole-cell current–voltage relationship determined in three neurons at this concentration. Like DAMGO, DPDPE produced a concentration-dependent reduction in EPSCs and was able to completely suppress EPSCs in two of four neurons (to 0% of control) when tested at 100 μM (Fig. 3*B*). Its EC_{50} (95% CL) was 5.7 (3.7–9.2) μM.

Application of 0.05–100 μM DELT also reversibly reduced EPSCs in a concentration-dependent manner. As with DPDPE, this reduction also often persisted much longer than the inhibition produced by an equieffective concentration of DAMGO (Fig. 2*C*). However, the reduction in EPSCs produced by DELT reached an asymptote of 50% of control at 10 μM. No further reduction was obtained even in the presence of 100 μM DELT (Fig. 3*C*). Thus, unlike either DAMGO or DPDPE, DELT was unable to completely suppress evoked EPSCs; the maximum inhibition observed in any one neuron was to 31% of control. The reduction in EPSCs was not accompanied by any appre-

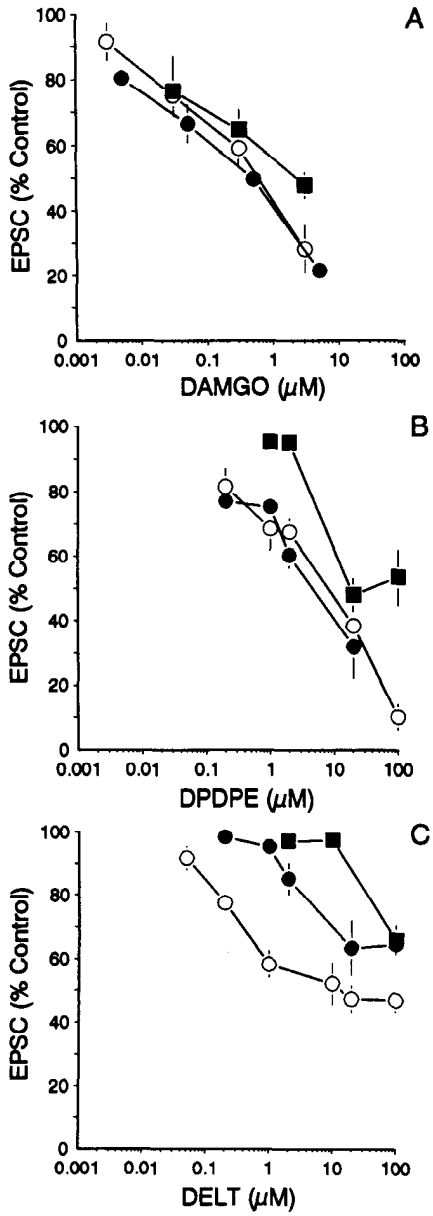

Figure 3. Concentration–effect relationships of, *A*, DAMGO; *B*, DPDPE; and *C*, DELT in the absence and presence of NTB, a δ_2 receptor antagonist. *Ordinate*, evoked EPSC or manually voltage-clamped EPSP expressed as percentage of control (see Materials and Methods); *abscissa*, concentration of agonist (μM). *Open circles*, agonist alone; *solid circles*, agonist in the presence of 200 nM NTB; *solid squares*, agonist in the presence of 1 μM NTB. Concentration–effect relationships were obtained by brief application of the agonist during continuous application of NTB. Symbols indicate the mean ± SEM. Where the SEM is not visible, it is encompassed by the symbol. In order of ascending concentration, the number of neurons tested with DAMGO alone was 3, 3, 9, and 10; with DAMGO and 200 nM NTB was 5, 5, 5, and 6; with DAMGO and 1 μM NTB was 3, 4, and 6; for DPDPE alone was 3, 7, 9, 5, and 4; for DPDPE and 200 nM NTB was 5, 7, 5, and 6; for DPDPE and 1 μM NTB was 5, 4, 6, and 5; for DELT alone was 6, 7, 6, 5, 9, 3; for DELT and 200 nM NTB was 3, 7, 4, 4, and 4; and for DELT and 1 μM NTB was 5, 4, and 4.

ciable changes in holding current (defined as >5 pA) even at the highest concentration of DELT tested. The EC_{75} (95% CL) of DELT was 0.3 (0.1–0.5) μM.

The site of action of DAMGO, DPDPE, or DELT was further defined in an ancillary series of experiments that examined the effects of these opioid receptor agonists on the postsynaptic current evoked by brief pressure ejection of (*S*)-AMPA. As illustrated in Figure 4, concentrations of DAMGO, DPDPE, or DELT that were able to suppress evoked EPSCs to 40–60% of control produced no change in the amplitude of the inward current produced by pressure ejection of (*S*)-AMPA. Thus, in the presence of 1 μM DAMGO, 2 μM DPDPE, or 1 μM DELT the amplitude of the AMPA-mediated current was 102.2 ± 7.1% (n = 5), 101.3 ± 3.3% (n = 6), and 101.1 ± 3.1% (n = 5) of control, respectively.

Effect of NTB on opioid inhibition of EPSCs

Bath application of 200 nM NTB selectively antagonized DELT, but not DAMGO or DPDPE (Fig. 3). In the presence of 200 nM NTB, the concentration–effect relationship of DELT was shifted to the right in a parallel manner and its EC_{75} (95% CL) was increased by 15.3-fold to 4.6 (3.0–8.4) μM (Fig. 3*C*; $P < 0.01$). By comparison, the EC_{50} (95% CL) for DAMGO or DPDPE in the presence of 200 nM NTB did not differ from that determined for each agonist alone [DAMGO: 0.3 (0.2–0.4) μM; DPDPE: 5.0 (2.7–12.7) μM]. A higher concentration of NTB, 1 μM, shifted the concentration–effect relationship of DELT further right in a parallel manner and increased the EC_{75} (95% CL) of DELT by 169.7-fold to 50.9 (32.9–92.9) μM (Fig. 3*C*; $P < 0.01$). This high concentration of NTB also produced a modest (threefold) rightward shift in the concentration–effect relationship of DPDPE (estimated EC_{50} 18 μM) that was accompanied by a marked decrease in the maximum effect produced by DPDPE, suggestive of noncompetitive antagonism. A similar noncompetitive antagonism was observed with DAMGO. Although 1 μM NTB did not antagonize the inhibition of EPSCs produced by 0.03 or 0.3 μM DAMGO, it did antagonize the inhibition produced by 3.0 μM DAMGO. Application of 1 μM NTB, the highest concentration tested, was by itself without effect and did not either increase or decrease the amplitude of the evoked EPSCs.

Effect of BNTX on opioid inhibition of EPSCs

Bath application of 33 nM BNTX produced a sixfold rightward shift in the concentration–effect relationship of DPDPE. The estimated EC_{50} (95% CL) for DPDPE was increased to 33.7 (11.3–345) μM. However, this shift was also accompanied by a marked reduction in the maximal effect produced by the two highest doses of DPDPE, indicative of noncompetitive antagonism (Fig. 5*B*). Increasing the concentration of BNTX to 100 nM resulted in a greater antagonism of DPDPE. Although an EC_{50} value for DPDPE could not be calculated, it appears to be increased by at least 17-fold (EC_{50} > 100 μM). By comparison, 100 nM BNTX did not antagonize the inhibition of EPSCs produced by DELT (Fig. 5*C*), but rather caused a slight, leftward shift in the concentration–effect relationship of this δ_2 receptor agonist, decreasing its EC_{75} (95% CL) to 0.1 (0.01–0.2) μM. However, this concentration of BNTX did appear to noncompetitively antagonize DAMGO (Fig. 5*A*). Addition of 100 nM BNTX, the highest concentration tested, by itself was without effect and neither increased nor decreased the amplitude of the evoked EPSCs.

Effect of naloxone on opioid inhibition of EPSCs

The effects of naloxone, a well-established competitive opioid receptor antagonist with approximately 10-fold selectivity for

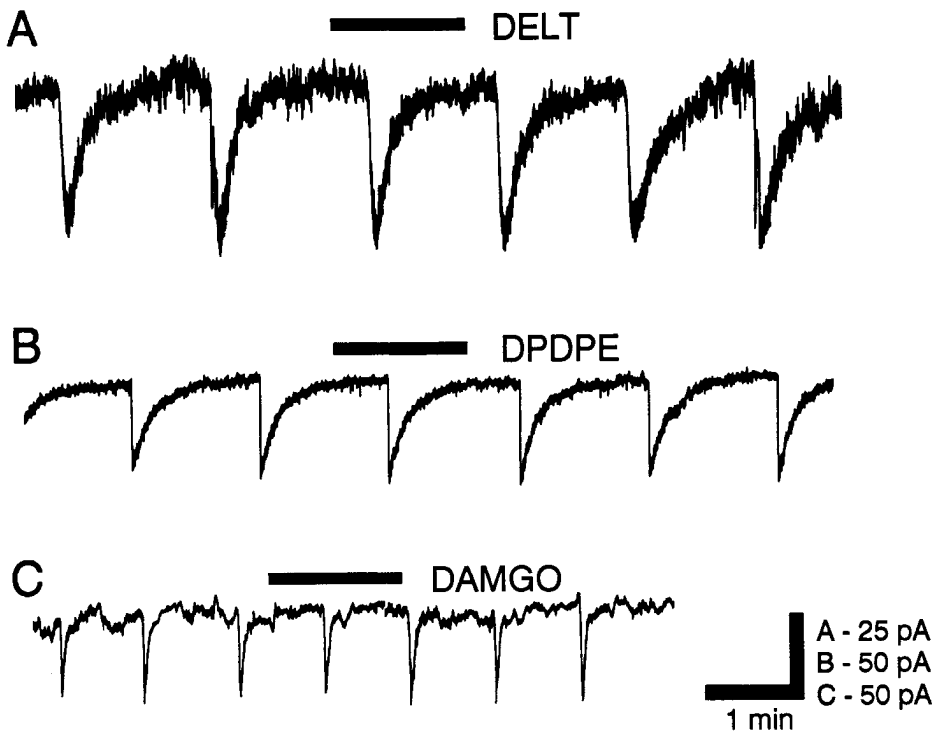

Figure 4. The postsynaptic inward current evoked by brief pressure ejection of the glutamate receptor agonist (*S*)-AMPA (1 mM, 12–25 msec, 4–6 psi) was unaltered in the presence of bath applied DELT (1 μM, *A*), DPDPE (2 μM, *B*), or DAMGO (1 μM, *C*). Currents in *A–C* were evoked in neurons in separate slices in the presence of 0.5 μM TTX, 10 μM bicuculline, 10 μM strychnine, and 50 μM D-2-amino-5-phosphonopentanoic acid. V_{hold}: *A*, −60 mV; *B*, −60 mV; *C*, −50 mV.

the μ receptor (Smith and Leslie, 1993), were also assessed to establish that competitive antagonism of DPDPE or DAMGO could be detected in this preparation. Bath application of 500 nM naloxone produced a parallel rightward shift in the concentration–effect relationships of DAMGO (Fig. 5*A*) and DPDPE (Fig. 5*B*), a finding consistent with competitive antagonism. The EC_{50} (95% CL) of DAMGO was increased by 13.3-fold to 7.1 (5.0–10.5) μM, whereas the EC_{50} of DPDPE was increased by only 2.5-fold to 14.1 (10.2–20.3) μM in the presence of naloxone ($P < 0.01$; both agonists). The K_b of naloxone at the μ- and δ_1 receptors was estimated to be 40 and 334 nM, respectively. The effect of naloxone on DELT was not examined as competitive antagonism of DELT by NTB was observed.

Discussion

These results provide the first evidence that excitatory, glutamatergic afferent transmission in lamina II of the spinal cord is inhibited by δ-opioid receptors. This conclusion is based on the ability of either DPDPE or DELT to reduce the amplitude of evoked EPSCs recorded in visually identified lamina II neurons under conditions in which inhibitory transmission was blocked. Several observations suggest that this effect is most likely mediated by a presynaptic site of action. First, at concentrations that reduced evoked EPSCs by 40–60%, neither DPDPE nor DELT reduced the amplitude of postsynaptic currents produced by direct pressure ejection of (*S*)-AMPA. Second, no change occurred in holding currents even after administration of 100 μM DPDPE or DELT, suggesting that neither DPDPE nor DELT exert a direct, postsynaptic inhibitory effect on lamina II neurons. By comparison, although 1.0 μM DAMGO did not reduce the amplitude of (*S*)-AMPA–mediated postsynaptic currents, bath application of 3.0 μM DAMGO produced outward currents in 4 of 10 neurons in the present study, in agreement with previous studies that used conventional intracellular electrodes and reported a direct postsynaptic inhibitory action of μ receptor agonists on dorsal horn neurons (Murase et al., 1982; Yoshimura and North, 1983; Jeftinija, 1988). Thus, it should have been possible to observe direct effects of either DPDPE or DELT on membrane current had these occurred. Finally, no desensitization occurred upon repeated application of either δ receptor agonist. Several investigators have noted the occurrence of rapid desensitization to the postsynaptic, but not the presynaptic inhibitory effects of opioid agonists (Jiang and North, 1992; Rhim et al., 1993).

The present results also provide the first electrophysiological evidence for the existence of functional subtypes of the δ receptor in the spinal cord of the rat. In this study, DPDPE and DELT each suppressed evoked EPSCs. However, they differed with respect to the magnitude of this effect. DPDPE produced a concentration-dependent reduction in evoked EPSCs and, at 100 μM, was able to completely suppress the evoked EPSCs. In contrast, the reduction in evoked EPSCs produced by DELT reached an asymptote of 50% of control at 10 μM. Although such effects could occur if DELT was a partial agonist at the same receptor at which DPDPE was a full agonist, this explanation is unlikely as the effects of DPDPE and DELT were differentially antagonized by NTB and BNTX. These observations indicate that selective activation of either δ_1 or δ_2 receptors inhibits excitatory, glutamatergic afferent transmission in the spinal cord.

Previous *in vitro* and *in vivo* investigations suggest that NTB and BNTX are competitive receptor antagonists (Portoghese et al., 1991, 1992; Sofuloglu et al., 1991, 1993). The parallel rightward shifts produced by NTB in the concentration–effect relationship of DELT are consistent with this conclusion. Estimates of the K_b of NTB in this preparation ranged from 5 nM (using 1 μM data) to 14 nM (using 200 nM data). The close agreement of these independent estimates also supports a conclusion of competitive antagonism (Kenakin, 1993). Nonetheless, there did appear to be some loss of efficacy of the highest concentrations of DELT in the presence of NTB, suggesting a modest

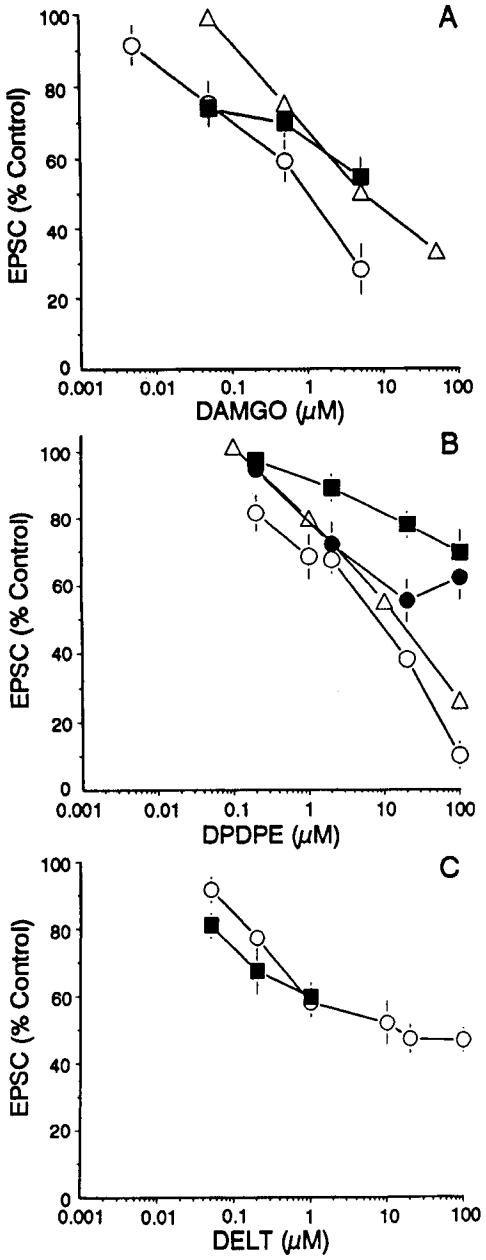

Figure 5. Concentration–effect relationships of, *A*, DAMGO; *B*, DPDPE; and *C*, DELT in the absence and presence of BNTX, a δ_1 receptor antagonist, or naloxone. *Ordinate,* evoked EPSC or manually voltage-clamped EPSP expressed as percentage of control (see Materials and Methods); *abscissa,* concentration of agonist (μM). *Open circles,* agonist alone; *solid circles,* agonist in the presence of 33 nM BNTX; *solid squares,* agonist in the presence of 100 nM BNTX; *open triangles,* agonist in the presence of 500 nM naloxone. Concentration–effect relationships were obtained by brief application of the agonist during continuous application of BNTX. Symbols indicate the mean ± SEM. Where the SEM is not visible, it is encompassed by the symbol. In order of ascending concentration, the numbers of neurons tested with DAMGO and 100 nM BNTX were 7, 5, and 5; with DAMGO and 500 nM naloxone were 5, 5, 5, and 5, with DPDPE and 33 nM BNTX were 4, 4, 4, and 5; with DPDPE and 100 nM BNTX were 5, 5, 5, and 5; with DPDPE and 500 nM naloxone were 5, 6, 5, and 3; and with DELT and 100 nM BNTX were 5, 5, and 5.

insurmountable antagonism. Our finding of noncompetitive, rather than competitive antagonism of DPDPE by BNTX is at odds with previous reports, but is strengthened by our finding that naloxone competitively antagonized DPDPE as well as DAMGO in this preparation. Not unexpectedly, high concentrations of both NTB and BNTX antagonized DAMGO. Both NTB and BNTX exhibit μ receptor antagonist activity at concentrations 100-fold and 4-fold greater, respectively, than their K_b for the δ receptor (Portoghese et al., 1991, 1992). Finally, although both BNTX and NTB interact competitively with the δ binding site, *functional* characterization of the antagonist activities of these drugs at μ- and δ-opioid receptors has been restricted to examinations of a single, optimally selective concentration or dose (Portoghese et al., 1991, 1992; Sofuloglu et al., 1991, 1993). Although these concentrations produce parallel shifts in the dose–effect relationships of δ- and μ-opioid receptor agonists that are compatible with competitive antagonism, the limited selectivity of these drugs and the agonist activity encountered at higher doses has precluded performance of the Schild analyses necessary for definitive characterization of the competitive nature of the antagonism.

Previous studies of the effects of opioid receptor agonists in slices of rat spinal cord concluded that excitatory afferent transmission was modulated predominantly by the μ-opioid receptor (Jeftinija, 1988; Hori et al., 1992). However, the ability of either DPDPE or DELT to reduce evoked EPSCs in a concentration-dependent, pharmacologically specific manner in the rat spinal cord indicates that δ receptors also modulate excitatory afferent transmission. One possible explanation for the apparent discrepancy between these results and those of earlier investigations is the age of the animals. Slices of spinal cord obtained from neonatal or juvenile rats were used in many of the previous studies (Murase et al., 1982; Jeftinija, 1988; Hori et al., 1992). However, the first 3 postnatal weeks is characterized by continued development and maturation of somatosensory pathways in the spinal cord of the rat. For example, C fibers are the last primary afferent fibers to innervate the spinal cord, entering the dorsal horn on or about the day of birth (Fitzgerald, 1985, 1987), and the reflexes subserved by polymodal C-fibers are not fully developed until nearly 3 weeks of age (Fitzgerald and Gibson, 1984). Furthermore, neurons in lamina II do not complete their differentiation and maturation processes until 3 weeks of age (Bicknell and Beal, 1984). Finally, in contrast to μ-opioid receptors, δ receptors in the brain and spinal cord do not appear until after birth and may not reach adult levels until nearly 3 weeks of age (Spain et al., 1985; McDowell and Kitchen, 1986; James et al., 1990; but see Attali et al., 1990). Thus, the results of previous studies may have been influenced by the use of neonatal or juvenile rats.

In conclusion, these results provide strong evidence that activation of either δ_1- or δ_2-opioid receptors inhibits excitatory, glutamatergic afferent transmission in the spinal cord of the rat. These results are also in excellent agreement with earlier *in vivo* investigations of spinal δ receptors in which intrathecal administration of DPDPE or DELT produced antinociception that was differentially antagonized by NTB and BNTX (Mattia et al., 1992; Sofuoglu et al., 1991, 1993; Stewart and Hammond, 1993). Thus, inhibition of excitatory, glutamatergic afferent transmission in the dorsal horn may mediate the ability of intrathecally administered δ_1 or δ_2 receptor agonists to produce antinociception.

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