Synergy between \( \mu / \delta \)-opioid receptors mediates adenosine release from spinal cord synaptosomes

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Abstract

Morphine releases adenosine from the spinal cord and this contributes to spinal antinociception. The present study examined possible interactions between \( \mu \) and subclasses of \( \delta \)-opioid receptors in the release of adenosine. Nanomolar (\( 10^{-8}, 10^{-9} \) M) concentrations of morphine release adenosine from spinal cord synaptosomes under conditions of partial depolarization with elevated K\(^+\), and this component of release is mediated by activation of \( \mu \)-opioid receptors. Subnanomolar (\( 10^{-10}, 10^{-11} \) M) concentrations of the \( \mu \)-opioid receptor agonists morphine, \([N-\text{MePhe}^3,\text{O-Pro}^4]\)morphiceptin, and \([\text{d-Ala}^2,\text{N-Me-Phe}^4,\text{Gly}^5\text{-ol}]\)enkephalin (DAMGO) have minimal effects on the release of adenosine from the spinal cord. However, \([\text{D-Pen}^2,\text{D-Pen}^4]\)enkephalin (DPDPE), a \( \delta \)-opioid receptor agonist, and \([\text{d-Ala}^2,\text{Cys}^4]\)deltorphin, a \( \delta \)-opioid receptor agonist, at doses which exhibit no intrinsic effects (\( 10^{-8} \) and \( 10^{-9} \) M), shifted the dose-response curve for \( \mu \)-opioid receptor-evoked adenosine release to the left in a dose-dependent manner. DPDPE was more potent than \([\text{d-Ala}^2,\text{Cys}^4]\)deltorphin when combined with the highly selective \( \mu \)-opioid receptor agonist \([N-\text{MePhe}^3,\text{O-Pro}^4]\)morphiceptin, but these agents showed similar activity with the less selective agonists DAMGO and morphine. Simultaneous activation of \( \mu \)- and \( \delta \)-opioid receptors generates a synergistic release of adenosine from spinal cord synaptosomes. Although agonists for both \( \delta \)- and \( \delta \)-opioid receptor subtypes produce this response, the \( \delta \)-opioid receptor agonist is more potent at eliciting this effect when the most selective \( \mu \)-opioid receptor ligand is used.

Keywords: Adenosine; Opioid; Spinal cord; Synergy

1. Introduction

Three opioid receptor subtypes \( \mu \), \( \delta \) and \( \kappa \) are present in the superficial layers of the rat dorsal spinal cord as determined by binding studies (\( \mu > \delta > \kappa \)) (Besse et al., 1991, Stevens et al., 1991). Activation of \( \mu \)-opioid receptors is considered the predominant receptor subtype responsible for eliciting spinal antinociception (reviewed Dickenson, 1993). The spinal administration of morphine is an effective therapeutic agent in the control of pain, but its use can be limited by side effects such as urinary retention and respiratory depression (Cousins and Mather, 1984). Recently, there has been considerable interest in the possibility of synergistic actions between drugs as an alternative to a single drug therapy for providing pain relief (reviewed Solomon and Gebhart, 1994). This could provide the advantage of using lower doses of individual analgesic agents to maintain the desired analgesic effect, thus limiting the side effects associated with higher doses. Several studies have demonstrated that antinociception generated by intrathecal (i.t.) opioid ligands acting at \( \mu \)-opioid receptors can be enhanced by coadministration of \( \delta \)-opioid receptor ligands and this represents a synergistic interaction (Heyman et al., 1989; Jiang et al., 1990; Malmberg and Yaksh, 1992; Porreca et al., 1992).

The development of selective agonists for \( \delta \)-opioid receptors has led to characterization of the \( \delta \)-opioid receptor subtypes and their involvement in \( \mu / \delta \)-opioid receptor-induced antinociception. In rats, the i.t. administration of the \( \delta \)-opioid receptor agonist DPDPE ([\text{D-Pen}^2,\text{D-Pen}^4]\)enkephalin) seemed more effective than the \( \delta \)-opioid receptor subtype agonist \([\text{d-Ala}^2,\text{Leu}^5,\text{Cys}^6]\)enkephalin (DALCE) in modulating \( \mu \) (morphine, \([\text{d-Ala}^2,\text{N-Me-Phe}^4,\text{Gly}^5\text{-ol}]\)enkephalin (DAMGO) and \([\text{N-MePhe}^3,\text{O-Pro}^4]\)morphiceptin)-opioid antinociception in thermal threshold tests (Malmberg and Yaksh, 1992). In mice, both \([\text{d-Ala}^2,\text{D-Glu}^5]\)deltorphin, a selective \( \delta \)-opioid receptor agonist, and DPDPE are effective in potentiating \( \mu \)-opioid...
antinociception (Mattia et al., 1992). However, the use of selective δ-opioid receptor antagonists has led to the conclusion that the δ-opioid receptor subtype is responsible for the modulation of μ-opioid receptor-mediated antinociception in the mouse (Porreca et al., 1992).

A number of mechanisms have been implicated in spinal antinociception by morphine (reviewed Yaksh and Malmberg, 1994). The spinal release of adenosine contributes to spinal antinociception by morphine (reviewed Sawynok and Sweeney, 1989). Antinociception produced by i.t. administration of selective μ-opioid receptor agonists is mediated in part via adenosine as antinociception is attenuated by methylxanthines (DeLander et al., 1992; Cahill et al., 1995). In neurochemical experiments nanomolar concentrations of morphine and the selective μ-opioid receptor agonists [N-MePhe³, Pro⁴]morphiceptin and DAMGO but not δ-opioid receptor agonists, release adenosine from dorsal spinal cord synaptosomes (Cahill et al., 1995). In the present study, we have examined the possible association between μ-opioid receptors and δ-opioid receptor subtypes by determining whether low nanomolar concentrations can synergistically enhance the release of adenosine from rat dorsal spinal cord synaptosomes.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (250–325 g; Charles River, Quebec, Canada) were housed in groups of two. They were maintained on a 12/12 h light/dark cycle and were allowed free access to food and water. Experiments were carried out according to a protocol approved by the Animal Care Committee at Dalhousie University, Nova Scotia.

2.2. Adenosine release from spinal cord synaptosomes

Synaptosomes were prepared from the dorsal half of rat spinal cord as previously described (Cahill et al., 1993a). Synaptosomes (P2 fraction) were resuspended in Krebs-Henseleit medium a final concentration of 1.5–2.5 mg protein/ml. Aliquots of synaptosomes (350 μl) were immediately added to microfuge tubes containing the drugs to be investigated (final volume of 365 μl). All opioid agonists were prepared in Krebs-Henseleit medium. Tube contents were incubated at 37°C for 10 min, followed by centrifugation to terminate the release of adenosine. Two tubes were incubated to determine the adenosine released during the preparation of the synaptosomes and the basal release of adenosine in the absence of drugs. Following deproteination the samples were derivitized with chloroacetaldehyde to form etheno-adenosine, which was detected by high performance liquid chromatography with fluorescence detection.

Adenosine release was expressed as picomoles per milligram of protein per 10 minutes (pmol/mg protein/10 min). Evoked values were determined by subtracting the adenosine released in the absence of drugs from that released in the presence of drugs. All experiments determining the effects of μ- and δ-opioid receptor agonist combinations were performed in the presence of a partial depolarization with K⁺ (an additional 6 mM raising the total extracellular K⁺ to 10.7 mM). The addition of 6 mM K⁺ alone did not release adenosine.

2.3. Statistics

The statistical method used to evaluate synergistic interactions was the dose-addition model (Wessinger, 1986). By using an inactive dose of one drug (δ-opioid receptor agonist) while eliciting a dose-related function for the other, a left-ward shift in the dose-response curve for the second drug indicates synergy (reviewed Solomon and Gebhart, 1994). This method was chosen over isobolographic analysis because an ED₅₀ for the δ-opioid receptor agonists could not be obtained as even micromolar concentrations do not release adenosine.

![Fig. 1. Dose-dependent release of adenosine by morphine with selected inactive doses of δ₁-opioid receptor agonist DPDPE (panel A) and δ₂-opioid receptor agonist [d-Ala²,Cys²]deltorphin (DELT) (panel B). Values represent mean ± S.E.M. for n = 4. ** P < 0.01 compared to release by morphine alone, *** P < 0.01 compared to basal release. Basal values ranged from 189 to 223 pmol/mg protein/10 min.](image-url)
Statistical comparisons were made using analysis of variance with the Student-Newman-Keuls test for post-hoc comparisons.

2.4. Drugs

Drugs used in this study were obtained from the following sources: DPDPE and [N-MePhe$^3$,d-Pro$^4$]morphiceptin (Peninsula Laboratories, Belmont, CA, USA), DAMGO (Sigma, St. Louis, MO, USA), morphine sulphate (British Drug Houses, Ontario, Canada), [d-Ala$^2$,Cys$^4$]deltorphin kindly supplied by Dr Frank Porreca. All drugs were dissolved in 0.9% NaCl w/v.

3. Results

Subnanomolar concentrations of morphine ($10^{-10}$, $10^{-11}$ M) had little effect on the release of adenosine from spinal cord synaptosomes (Fig. 1A). Following the addition of the $\delta_1$-opioid receptor agonist DPDPE ($10^{-8}$ and $10^{-7}$ M), which by itself did not alter the release of adenosine, a modest enhancement of the release of adenosine occurred at $10^{-10}$ and $10^{-9}$ M morphine (Fig. 1A). The $\delta_2$-opioid receptor agonist [d-Ala$^2$,Cys$^4$]deltorphin when combined with morphine, produced a greater degree of enhancement of adenosine release (Fig. 1B).

Subnanomolar concentrations ($10^{-11}$, $10^{-10}$ M) of the selective $\mu$-opioid receptor agonist DAMGO had little effect on the release of adenosine (Fig. 2A). The addition of DPDPE significantly augmented the release of adenosine, as DAMGO now released adenosine at $10^{-10}$ M, and there was an augmentation of release at $10^{-9}$ M (Fig. 2A). DAMGO enhanced the release of adenosine when combined with [d-Ala$^2$,Cys$^4$]deltorphin in a similar manner (Fig. 2B).
Subnanomolar concentrations (10^{-11}, 10^{-10} \text{ M}) of the selective $\mu$-opioid receptor agonist $[\text{N-MePhe}^3,\text{D-Pro}^4] \text{morphiceptin}$ likewise did not release adenosine. DPDPE (10^{-8}, 10^{-7} \text{ M}) released adenosine in combination with 10^{-10} \text{ M} $[\text{N-MePhe}^3,\text{D-Pro}^4] \text{morphiceptin}$ and enhanced 10^{-9} \text{ M} $[\text{N-MePhe}^3,\text{D-Pro}^4] \text{morphiceptin}$ (Fig. 3A). $[\text{D-Ala}^2,\text{Cys}^4] \text{deltorphin}$ produced only a minimal enhancement of $[\text{N-MePhe}^3,\text{D-Pro}^4] \text{morphiceptin}$-mediated release of adenosine (Fig. 3B).

4. Discussion

We have recently demonstrated that $\mu$- but not $\delta$-selective opioid receptor agonists release adenosine from spinal cord synaptosomes at nanomolar concentrations (Cahill et al., 1995). Such neurochemical data are consistent with the observation that methylxanthines inhibit spinal antinociception produced by $\mu$-opioid receptor agonists (DeLander et al., 1992; Cahill et al., 1995) but not $\delta$-opioid receptor agonists (Cahill et al., 1995; but see DeLander et al., 1992). Spinal release of adenosine by morphine is capsaicin-sensitive implicating a primary afferent source for this release (Sweeney et al., 1989). The present study demonstrates that $\mu$- and $\delta$-opioid receptors interact to enhance the release of adenosine in a supra-additive manner. Both $\mu$- and $\delta$-opioid receptors have been identified on presynaptic afferent nerve terminals (Fields et al., 1980; Bess et al., 1991), providing anatomical support for an interaction between $\mu$- and $\delta$-opioid receptors in spinal cord synaptosomes. Both receptors also coexist on the cell body of dorsal root ganglion neurons (Shen and Crain, 1989). Behavioral experiments had previously provided evidence for a positive modulation of the antinociceptive action of $\mu$-opioid receptor agonists by $\delta$-opioid receptor ligands. Thus, the i.t. administration of DPDPE was shown to augment $\mu$-opioid receptor agonists (morphine, $[\text{N-MePhe}^3,\text{D-Pro}^4] \text{morphiceptin}$ and DAMGO) in both the thermal and pressure threshold tests (Sutters et al., 1990; Malmberg and Yaksh, 1992; Mattia et al., 1992; Miaskowski et al., 1992).

Ligand binding studies indicate that the degree of selectivity for $[\text{N-MePhe}^3,\text{D-Pro}^4] \text{morphiceptin}$ is $> 600$-fold, for DAMGO is $130$-fold and for morphine is $60$-fold greater for $\mu$-opioid receptors to $\delta$ (Chang et al., 1983; James and Goldstein, 1984; Cahill et al., 1995). DPDPE (780-fold-selective) and $[\text{D-Ala}^2,\text{Cys}^4] \text{deltorphin}$ (1000-fold-selective) have higher selectivity for the $\delta$-opioid receptor compared to the $\mu$-opioid receptor site (Mosberg et al., 1983; Erspamer et al., 1989). The degree of enhancement of adenosine release by each $\mu$-opioid receptor agonist varied for the two $\delta$-opioid receptor ligands. Both $\delta_1$- and $\delta_2$-opioid receptor subtypes produce a synergistic effect with $\mu$-opioid receptor agonists to release adenosine. However, the $\delta_2$-opioid receptor agonist DPDPE appears more potent than the $\delta_1$-opioid receptor agonist $[\text{D-Ala}^2,\text{Cys}^4] \text{deltorphin}$ in producing the $\mu/\delta$-opioid receptor synergy when combined with the most selective $\mu$-opioid receptor agonist $[\text{N-MePhe}^3,\text{D-Pro}^4] \text{morphiceptin}$. Less difference was exhibited when these agents were combined with the less selective $\mu$-opioid receptor agonists DAMGO and morphine. Consistent with these data is the report by Malmberg and Yaksh (1992) demonstrating that the magnitude of augmentation by DPDPE ($\delta_1$) by combination with $\mu$-opioid receptor agonists after i.t. administration in rats was $[\text{N-MePhe}^3,\text{D-Pro}^4] \text{morphiceptin} > \text{DAMGO} > \text{morphine}$. While the methylxanthine-sensitivity of antinociception produced by combinations of $\mu$ and $\delta$ opioid receptor agonists has not been determined, the present results suggest that the behavioral effects of such combinations should be reduced by methylxanthines. The high levels of adenosine release produced by micromolar concentrations of morphine may be attributed to an interaction between $\mu$- and $\delta$-opioid receptors which are simultaneously activated by morphine at these doses (Cahill et al., 1995).

The mechanism by which $\mu$-opioid receptor-mediated adenosine release is enhanced by $\delta$-opioid receptor activation is not clear, but a number of mechanisms are possible. These include receptor receptor allosteric interactions at the cell surface level or second messenger interactions (reviewed by Solomon and Gehhart, 1994). Functional interactions between receptors also can occur due to activation at different sites within the cascade of nociceptive integration (Malmberg and Yaksh, 1992; Traynor and Elliott, 1993), but this is unlikely to contribute to interactions observed at the synaptosomal level in the present study. There are some data suggesting that the $\mu/\delta$-opioid receptor-evoked release of adenosine from spinal cord synaptosomes is Ca^{2+}-dependent and involves activation of a N-type voltage-sensitive Ca^{2+} channel (Cahill et al., 1992), reflecting the properties of morphine (Cahill et al., 1993b). Nanomolar concentrations of $\mu$-opioid receptor agonists release adenosine per se from spinal cord synaptosomes (Cahill et al., 1995), and it is likely that the combination of $\delta$- with $\mu$-opioid receptor agonists has characteristics similar to the $\mu$-opioid receptor-mediated release of adenosine. In other systems, opioids increase intracellular Ca^{2+} levels via activation of the phosphoinositide cascade (Jin et al., 1992, 1994; Smart et al., 1994). Stimulation of protein kinase C has recently been shown to enhance K^+-evoked peptide release from primary afferent neurons (Barber and Vasko, 1994). It is possible that enhanced release of adenosine by opioids results from a similar mechanism.

In summary, inactive doses of either $[\text{D-Ala}^2,\text{Cys}^4] \text{deltorphin}$ or DPDPE act synergistically when combined with subnanomolar doses of morphine, $[\text{N-MePhe}^3,\text{D-Pro}^4] \text{morphiceptin}$ or DAMGO to enhance the release of adenosine from spinal cord synaptosomes, indicating that $\mu/\delta$-opioid receptor synergy is expressed at the synaptosomal level as well as in more integrated systems utilized in behavioral studies. This release occurs at much lower
doses of opioid receptor agonists than previously reported and thus may be one of the mechanisms contributing to the phenomenon of spinal antinociceptive synergy.

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References

Barber, L.A. and M.R. Vasko, 1994, Activation of protein kinase C increases the release of neuropeptides from rat sensory neurons, Soc. Neurosci. Abstr. 20, 964.

Besse, D., M.C. Lombard and J.M. Bessen, 1991, Autoradiographic distribution of mu, delta and kappa opioid binding sites in the superficial dorsal horn, over the rostrocaudal axis of the rat spinal cord, Brain Res. 548, 787

Cahill, C.M., T.D. White and J. Sawynok, 1992, Mu and mu/delta interactions mediate opioid-induced release of adenosine via N-type Ca²⁺ channels from dorsal spinal cord synaptosomes, Soc. Neurosci. Abstr. 18, 1371.

Cahill, C.M., T.D. White and J. Sawynok, 1993a, Involvement of calcium channels in depolarization-evoked release of adenosine from spinal cord synaptosomes, J. Neurochem. 60, 886.

Cahill, C.M., T.D. White and J. Sawynok, 1993b, Morphine activates ω-conotoxin-sensitive Ca²⁺ channels to release adenosine from spinal cord synaptosomes, J. Neurochem. 60, 894.

Cahill, C.M., T.D. White and J. Sawynok, 1995, Spinal opioid receptors and adenosine release: neurochemical and behavioral characterization of subtypes, J. Pharmacol. Exp. Ther. (in press).

Chang, K.-J., E.T. Wei, A. Killian and J.-K. Chang, 1983, Potent inhibitors of 6 opioid binding sites, Proc. Natl. Acad. Sci. USA 80, 5187.

Cousins, M.J. and L.E. Mather, 1984, Intrathecal and epidural administration of opioids, Anesthesiology 61, 276.

DeLander, G.E., H.I. Mosberg and F. Porreca, 1992, Involvement of adenosine in antinociception produced by spinal or supraspinal receptor-selective opioid agonists: dissociation from gastrointestinal effects in mice, J. Pharmacol. Exp. Ther. 263, 1097.

Dickenson, A.H., 1993, Where and how do opioids act?, in: Proceedings of the 7th World Congress on Pain, eds. G.F. Gebhart, D.L. Hammond and T.S. Jensen (IASP Press) p. 525.

Ersparme, V., P. Melchiorri, G. Falconieri-Ersparme, L. Negri, R. Corsi, C. Severini, D. Barra, M. Simmaco and G. Kreli, 1989, Deltorphin: a family of naturally occurring peptides with a high affinity and selectivity for δ opioid binding sites, Proc. Natl. Acad. Sci. USA 86, 5188.

Fields, H.L., P.C. Einson, B.K. Keigh, R.F.T. Gilbert and L.L. Iverson, 1980, Multiple opioid receptors on primary afferent fibers, Nature 284, 351.

Goldstein, A., 1987, Binding selectivity profiles for ligands of multiple receptor types: focus on opioid receptors, Trends Pharmacol. Sci. 8, 456.

Heyman, J.S., Q. Jiang, R.B. Rothman, H.I. Mosberg and F. Porreca, 1989, Modulation of μ-mediated antinociception by δ opioid agonists: characterization with antagonists, Eur. J. Pharmacol. 169, 43.

James, I.F. and A. Goldstein, 1984, Site-directed alkylation of multiple opioid receptors, Mol. Pharmacol. 25, 337.

Jiang, Q., H.I. Mosberg and F. Porreca, 1990, Modulation of the potency and efficacy of μ-mediated antinociception by delta agonists in the mouse, J. Pharmacol. Exp. Ther. 254, 685.

Jin, W., N.M. Lee, H.H. Loh and S.A. Thayer, 1992, Dual excitatory and inhibitory effects of opioids on intracellular calcium in neuroblastoma X glioma hybrid NG108-15 cells, Mol. Pharmacol. 42, 1083.

Jin, W., N.M. Lee, H.H. Loh and S.A. Thayer, 1994, Opioids mobilize calcium from 1,4,5-trisphosphate-sensitive stores in NG108-15 cells, J. Neurosci. 14, 1920.

Malmberg, A.B. and T.L. Yaksh, 1992, Isobolographic and dose-response analysis of the interaction between intrathecal mu and delta agonists: effects of naltrindole and its benzofuran analog (NTB), J. Pharmacol. Exp. Ther. 263, 764.

Mattia, A., S.C. Farmer, A.E. Takemori, M. Sultana, P.S. Portoghese, H.I. Mosberg, W.D. Bowen and F. Porreca, 1992, Spinal opioid delta antinociception in the mouse: meditation by a single subtype of opioid delta receptor, J. Pharmacol. Exp. Ther. 260, 518.

Miaskowski, C., K.A. Sutters, Y.O. Taiwo and J.D. Levine, 1992, Antinociceptive and motor effects of delta/mu and kappa/mu combinations of intrathecal opioid agonists, Pain 49, 137.

Mosberg, H.I., R. Horst, V.J. Hruby, K. Gee, H.I. Yamamura, J.J. Galligan and T.F. Burks, 1983, Bis-penicillamine enkephalins possess highly improved specificity towards δ opioid receptors, Proc. Natl. Acad. Sci. USA 80, 5871.

Porreca, F., A.E. Takemori, M. Sultana, P.S. Portoghese, W.D. Bowen and H.I. Mosberg, 1992, Modulation of mu-mediated antinociception in the mouse involves opioid delta-2 receptors, J. Pharmacol. Exp. Ther. 263, 147.

Sawynok, J. and M.I. Sweeney, 1989, The role of purines in nociception, Neuroscience 32, 557.

Shen, K.E. and S.M. Crain, 1989, Dual opioid modulation of the action potential duration of mouse dorsal root ganglion neurons in culture, Brain Res. 491, 227.

Smart, D., G. Smith and D.G. Lambert, 1994, μ-Opioid receptor stimulation of inositol (1,4,5)trisphosphate formation via a pertussis toxin-sensitive G protein, J. Neurochem. 62, 1009.

Solomon, R.E. and G.F. Gebhart, 1994, Synergistic antinociceptive interactions among drugs administered to the spinal cord, Anesth. Analg. 78, 1164.

Stevens, C.W., C.B. Lacey, K.E. Miller, R.P. Elde and V.S. Seybold, 1991, Biochemical characterization and regional quantification of mu, delta and kappa opioid binding sites in rat spinal cord, Brain Res. 550, 77.

Sutters, K.A., C. Miaskowski, Y.O. Taiwo and J.D. Levine, 1990, Analgesic synergy and improved motor function produced by combinations of mu-delta and mu-kappa- opioids, Brain Res. 530, 290.

Sweeney, M.I., T.D. White, J. Sawynok, 1989, Morphine, capsaicin and K⁺ release purines from capsaicin-sensitive primary afferent nerve terminals in the spinal cord, J. Pharmacol. Exp. Ther. 248, 447.

Traynor, J.R. and J. Elliot, 1993, δ-Opioid receptor subtypes and cross-talk with μ-receptors, Trends Pharmacol. Sci. 14, 84.

Wessinger, W.D., 1986, Approaches to the study of drug interactions in behavioral pharmacology, Neurosci. Biobehav. Rev. 10, 103.

Yaksh, T.L. and A.B. Malmberg, 1994, Central pharmacology of nociceptive transmission, in: Textbook of Pain, 3rd edn., eds. P.D. Wall and R. Melzack (Churchill Livingstone) p. 165.

James, I.F. and A. Goldstein, 1984, Site-directed alkylation of multiple opioid receptors, Mol. Pharmacol. 25, 337.

Jin, W., N.M. Lee, H.H. Loh and S.A. Thayer, 1992, Dual excitatory and inhibitory effects of opioids on intracellular calcium in neuroblastoma X glioma hybrid NG108-15 cells, Mol. Pharmacol. 42, 1083.

Jin, W., N.M. Lee, H.H. Loh and S.A. Thayer, 1994, Opioids mobilize calcium from 1,4,5-trisphosphate-sensitive stores in NG108-15 cells, J. Neurosci. 14, 1920.

Malmberg, A.B. and T.L. Yaksh, 1992, Isobolographic and dose-response analysis of the interaction between intrathecal mu and delta agonists: effects of naltrindole and its benzofuran analog (NTB), J. Pharmacol. Exp. Ther. 263, 764.