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Synergy between μ/δ -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 μ - and subclasses of δ -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 μ -opioid receptors. Subnanomolar (10^{-10} , 10^{-11} M) concentrations of the μ -opioid receptor agonists morphine, [*N*-MePhe³,D-Pro⁴]morphiceptin, and [D-Ala²,*N*-Me-Phe⁴,Gly⁵-ol]enkephalin (DAMGO) have minimal effects on the release of adenosine from the spinal cord. However, [D-Pen²,D-Pen⁵]enkephalin (DPDPE), a δ_1 -opioid receptor agonist, and [D-Ala²,Cys⁴]deltorphin, a δ_2 -opioid receptor agonist, at doses which exhibit no intrinsic effects (10^{-8} and 10^{-7} M), shifted the dose-response curve for μ -opioid receptor-evoked adenosine release to the left in a dose-dependent manner. DPDPE was more potent than [D-Ala²,Cys⁴]deltorphin when combined with the highly selective μ -opioid receptor agonist [*N*-MePhe³,D-Pro⁴]morphiceptin, but these agents showed similar activity with the less selective agonists DAMGO and morphine. Simultaneous activation of μ - and δ -opioid receptors generates a synergistic release of adenosine from spinal cord synaptosomes. Although agonists for both δ_1 - and δ_2 -opioid receptor subtypes produce this response, the δ_1 -opioid receptor agonist is more potent at eliciting this effect when the most selective μ -opioid receptor ligand is used.

Keywords: Adenosine; Opioid; Spinal cord; Synergy

1. Introduction

Three opioid receptor subtypes μ , δ and κ 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 μ -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 μ -opioid receptors can be enhanced by coadministration of δ -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 δ -opioid receptors has led to characterization of the δ -opioid receptor subtypes and their involvement in μ/δ -opioid receptor-induced antinociception. In rats, the i.t. administration of the δ_1 -opioid receptor agonist DPDPE ([D-Pen²,D-Pen⁵]enkephalin) seemed more effective than the δ_2 -opioid receptor subtype agonist [D-Ala²,Leu⁵,Cys⁶]enkephalin (DALCE) in modulating μ (morphine, [D-Ala²,*N*-Me-Phe⁴,Gly⁵ol]enkephalin (DAMGO) and [*N*-MePhe³,D-Pro⁴]morphiceptin)-opioid antinociception in thermal threshold tests (Malmberg and Yaksh, 1992). In mice, both (D-Ala²,D-Glu⁴)deltorphin, a selective δ_2 -opioid receptor agonist, and DPDPE are effective in potentiating μ -opioid

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antinociception (Mattia et al., 1992). However, the use of selective δ -opioid receptor antagonists has led to the conclusion that the δ_2 -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,*D*-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 μ l) were immediately added to microfuge tubes containing the drugs to be investigated (final volume of 365 μ l). 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 mil-

ligram 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 ED_{50} for the δ -opioid receptor agonists could not be obtained as even micromolar concentrations do not release adenosine.

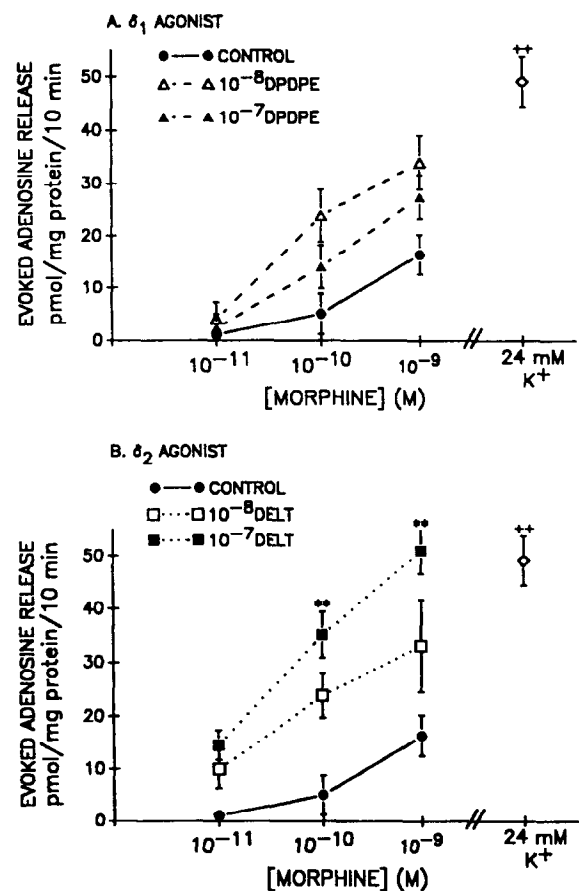


Fig. 1. Dose-dependent release of adenosine by morphine with selected inactive doses of δ_1 -opioid receptor agonist DPDPE (panel A) and δ_2 -opioid receptor agonist [D-Ala²,Cys⁴]deltorphin (DELTA) (panel B). Values represent means \pm S.E.M. for $n = 4$. ** $P < 0.01$ compared to release by morphine alone, + $P < 0.01$ compared to basal release. Basal values ranged from 189 to 223 pmol/mg protein/10 min.

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*-MePhe³,D-Pro⁴]morphiceptin (Peninsula Laboratories, Belmont, CA, USA), DAMGO (Sigma, St. Louis, MO, USA), morphine sulphate (British Drug Houses, Ontario, Canada), [*D*-Ala²,Cys⁴]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

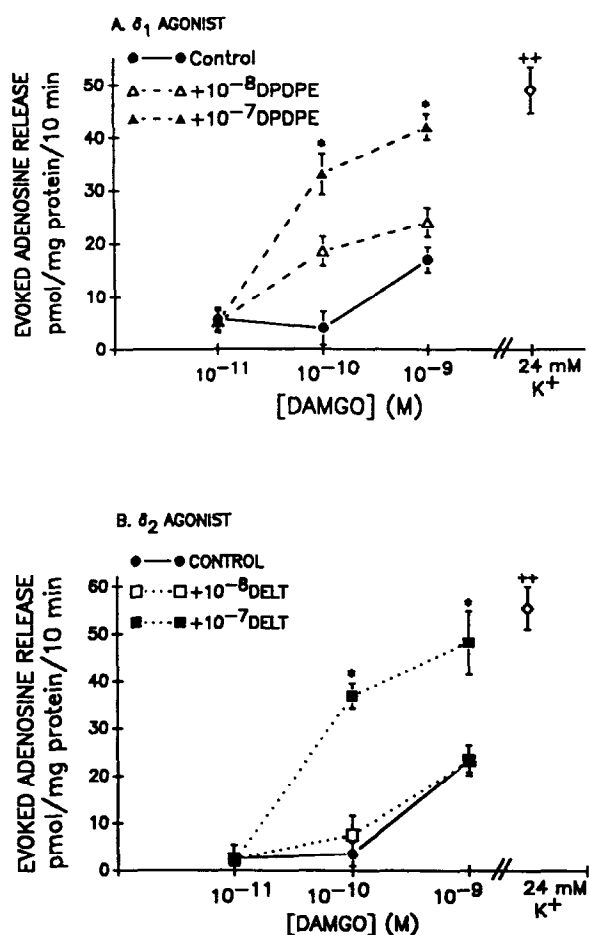


Fig. 2. Dose-dependent release of adenosine by DAMGO with selected inactive doses of δ_1 -opioid receptor agonist DPDPE (panel A) and δ_2 -opioid receptor agonist [*D*-Ala²,Cys⁴]deltorphin (DELTA) (panel B). Values represent means \pm S.E.M. for $n = 4$. * $P < 0.05$ compared to DAMGO alone, ** $P < 0.01$ compared to basal release. Basal values ranged from 210 to 230 pmol/mg protein/10 min.

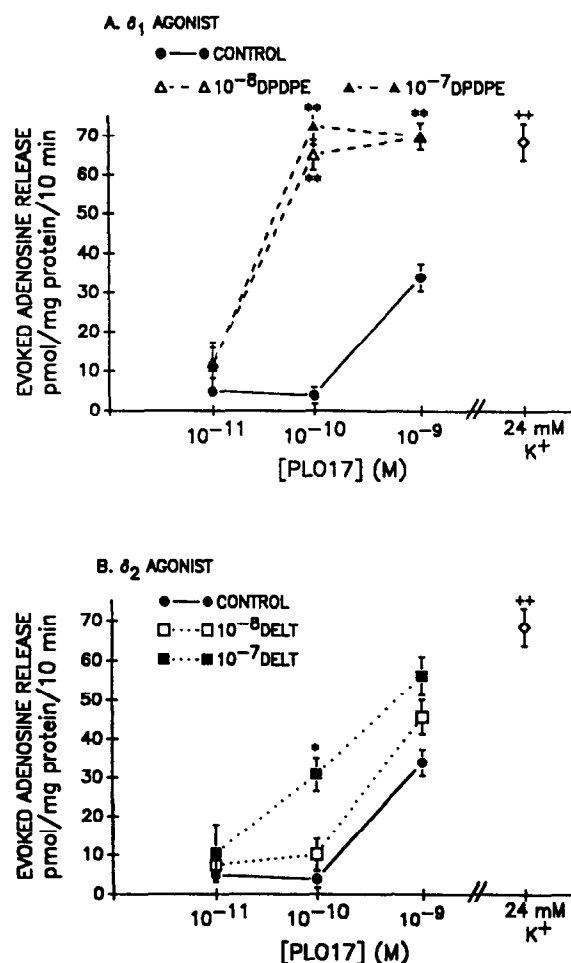


Fig. 3. Dose-dependent release of adenosine by [*N*-MePhe³,D-Pro⁴]morphiceptin (PLO17) with selected inactive doses of δ_1 -opioid receptor agonist DPDPE (panel A) and δ_2 -opioid receptor agonist [*D*-Ala²,Cys⁴]deltorphin (DELTA) (panel B). Values represent means \pm S.E.M. for $n = 4$. * $P < 0.05$, ** $P < 0.01$ compared to [*N*-MePhe³,D-Pro⁴]morphiceptin alone, ** $P < 0.01$ compared to basal release. Basal values ranged from 202 to 229 pmol/mg protein/10 min.

spinal cord synaptosomes (Fig. 1A). Following the addition of the δ_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 δ_2 -opioid receptor agonist [*D*-Ala²,Cys⁴]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 μ -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*-Ala²,Cys⁴]deltorphin in a similar manner (Fig. 2B).

Subnanomolar concentrations (10^{-11} , 10^{-10} M) of the selective μ -opioid receptor agonist [*N*-MePhe³,D-Pro⁴]morphiceptin likewise did not release adenosine. DPDPE (10^{-8} , 10^{-7} M) released adenosine in combination with 10^{-10} M [*N*-MePhe³,D-Pro⁴]morphiceptin and enhanced 10^{-9} M [*N*-MePhe³,D-Pro⁴]morphiceptin (Fig. 3A). [D-Ala²,Cys⁴]deltorphin produced only a minimal enhancement of [*N*-MePhe³,D-Pro⁴]morphiceptin-mediated release of adenosine (Fig. 3B).

4. Discussion

We have recently demonstrated that μ - but not δ -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 μ -opioid receptor agonists (DeLander et al., 1992; Cahill et al., 1995) but not δ -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 μ - and δ -opioid receptors interact to enhance the release of adenosine in a supra-additive manner. Both μ - and δ -opioid receptors have been identified on presynaptic afferent nerve terminals (Fields et al., 1980; Besse et al., 1991), providing anatomical support for an interaction between μ - and δ -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 μ -opioid receptor agonists by δ -opioid receptor ligands. Thus, the i.t. administration of DPDPE was shown to augment μ -opioid receptor agonists (morphine, [*N*-MePhe³,D-Pro⁴]morphiceptin and DAMGO) in both the thermal and pressure threshold tests (Sutters et al., 1990; Malmberg and Yaksh, 1992; Mattia et al., 1992; Miskowski et al., 1992).

Ligand binding studies indicate that the degree of selectivity for [*N*-MePhe³,D-Pro⁴]morphiceptin is > 600-fold, for DAMGO is 130-fold and for morphine is 60-fold greater for μ -opioid receptors compared to δ (Chang et al., 1983; James and Goldstein, 1984; Goldstein, 1987). DPDPE (780-fold-selective) and [D-Ala²,Cys⁴]deltorphin (1000-fold-selective) have higher selectivity for the δ -opioid receptor compared to the μ -opioid receptor site (Mosberg et al., 1983; Erspamer et al., 1989). The degree of enhancement of adenosine release by each μ -opioid receptor agonist varied for the two δ -opioid receptor ligands. Both δ_1 - and δ_2 -opioid receptor subtypes produce a synergistic effect with μ -opioid receptor agonists to release adenosine. However, the δ_1 -opioid receptor agonist DPDPE appears more potent than the δ_2 -opioid receptor

agonist [D-Ala²,Cys⁴]deltorphin in producing the μ/δ -opioid receptor synergy when combined with the most selective μ -opioid receptor agonist [*N*-MePhe³,D-Pro⁴]morphiceptin. Less difference was exhibited when these agents were combined with the less selective μ -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 (δ_1) by combination with μ -opioid receptor agonists after i.t. administration in rats was [*N*-MePhe³,D-Pro⁴]morphiceptin > DAMGO > morphine. While the methylxanthine-sensitivity of antinociception produced by combinations of μ - and δ -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 μ - and δ -opioid receptors which are simultaneously activated by morphine at these doses (Cahill et al., 1995).

The mechanism by which μ -opioid receptor-mediated adenosine release is enhanced by δ -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 Gebhart, 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 Elliot, 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 μ/δ -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 μ -opioid receptor agonists release adenosine per se from spinal cord synaptosomes (Cahill et al., 1995), and it is likely that the combination of δ - with μ -opioid receptor agonists has characteristics similar to the μ -opioid receptor-mediated release of adenosine. In other systems, opioids increase intracellular Ca^{2+} levels via activation of the phosphoinositol 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 [D-Ala²,Cys⁴]deltorphin or DPDPE act synergistically when combined with subnanomolar doses of morphine, [*N*-MePhe³,D-Pro⁴]morphiceptin or DAMGO to enhance the release of adenosine from spinal cord synaptosomes, indicating that μ/δ -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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