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Authors

Cahill, CM
White, TD
Sawynok, J

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Morphine Activates ω -Conotoxin-Sensitive Ca^{2+} Channels to Release Adenosine from Spinal Cord Synaptosomes

C. M. Cahill, T. D. White, and J. Sawynok

Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada

Abstract: Morphine-induced release of adenosine from the spinal cord is believed to contribute to spinal antinociception. Although this release is Ca^{2+} dependent, little is known of the nature of this dependence. In this study, the effects of the dihydropyridine L-type Ca^{2+} channel agonist Bay K 8644 and the antagonist nifedipine, the N-type Ca^{2+} channel antagonist ω -conotoxin, and ruthenium red, a blocker of Ca^{2+} influx induced by capsaicin, on release of adenosine evoked by morphine were determined. The effect of partial depolarization with a minimally effective concentration of K^+ on morphine-evoked release of adenosine also was examined. Morphine 10^{-5} – 10^{-4} M produced a dose-dependent enhancement of adenosine release from dorsal spinal cord synaptosomes. Following the addition of 6 mM K^+ (total K^+ concentration of 10.7 mM), 10^{-6} M morphine also enhanced release, and an additional component of action at 10^{-8} M was revealed. Release was Ca^{2+} -dependent as it was

not observed in the absence of Ca^{2+} and presence of EGTA. Bay K 8644 (10 nM) and nifedipine (100 nM) had no effect on the release of adenosine evoked by morphine, but ω -conotoxin (100 nM) markedly reduced such release in both the absence and the presence of the additional 6 mM K^+ . Morphine-evoked adenosine release was not altered in the presence of a partially effective dose of capsaicin, nor by ruthenium red. These results indicate that morphine can stimulate two distinct phases of adenosine release from the spinal cord (nanomolar and micromolar), and that both phases of release are due to Ca^{2+} entry via ω -conotoxin-sensitive N-type Ca^{2+} channels. **Key Words:** Morphine—Dihydropyridines — ω -Conotoxin — Adenosine — Synaptosomes — Spinal cord. **Cahill C. M. et al.** Morphine activates ω -conotoxin-sensitive Ca^{2+} channels to release adenosine from spinal cord synaptosomes. *J. Neurochem.* **60**, 894–901 (1993).

Within the spinal cord, opioids inhibit the release of peptides such as substance P (Jessell and Iversen, 1977; Yaksh et al., 1980; Lembeck and Donnerer, 1985; Pang and Vasko, 1986; Aimone and Yaksh, 1989) and calcitonin gene-related peptide (Pohl et al., 1989) from sensory nerve terminals. Morphine also has been shown to release adenosine from capsaicin-sensitive afferent nerve terminals in the spinal cord (Sweeney et al., 1987, 1989). Both inhibition of the release of substance P (which promotes nociception; Otsuka and Yanagisawa, 1987) and augmentation of the release of adenosine (which inhibits nociception; Sawynok and Sweeney, 1989) may contribute to spinal antinociception by morphine (Yaksh and Noueihed, 1985; Sawynok and Sweeney, 1989; Dickenson, 1991).

An understanding of the dual effects of opioids on neurotransmitter/neuromodulator release from the

spinal cord has become apparent in the last few years. Thus, opioids exert biphasic effects on the action potential duration of cultured dorsal root ganglion cells, augmenting it at low nanomolar doses and inhibiting it at higher micromolar doses (Crain et al., 1988; Shen and Crain, 1989; Crain and Shen, 1990). Both inhibitory (Mudge et al., 1979; Werz and MacDonald, 1983) and excitatory (Shen and Crain, 1989; Crain and Shen, 1990) modulations of action potential duration result from effects on voltage-sensitive K^+ and Ca^{2+} channels. Morphine has been shown to inhibit Ca^{2+} uptake into nerve terminals from central neurons (Guerrero-Munoz et al., 1979; End et al., 1981; Kamikubo et al., 1983; Bradford et al., 1986). Both the inhibition of action potential duration and the decrease in Ca^{2+} uptake contribute to inhibition of neurotransmitter/neuromodulator release from the spinal cord. Prolongation of the action potential dura-

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Address correspondence and reprint requests to Dr. J. Sawynok

at Department of Pharmacology, Tupper Medical Building, Dalhousie University, Halifax, Nova Scotia B3H 4H7, Canada.

tion by opioids has been proposed as the basis of augmentation of neurotransmitter/neuromodulator release (Crain and Shen, 1990). It is interesting that there are also reports that opioids can increase Ca^{2+} uptake into synaptosomes from certain brain regions (Barr and Leslie, 1985) and into neuroblastoma and hybrid cells (Lorentz et al., 1988; Jin et al., 1991), raising the possibility that enhanced Ca^{2+} entry into nerve terminals contributes to such enhancement.

Ca^{2+} entry into cells, with a consequent change in free cytosolic Ca^{2+} levels and alterations in cellular response, occurs via three types of voltage-sensitive Ca^{2+} channels: L-, N-, and T-type (Nowicky et al., 1985; Augustine et al., 1987; Miller, 1987). L-type Ca^{2+} channels are modulated by dihydropyridine agonists and antagonists, whereas N-type Ca^{2+} channels are selectively blocked by ω -conotoxin (Tsien et al., 1988; Sher and Clementi, 1991). Both L-type (Perney et al., 1986; Holz et al., 1988) and N-type Ca^{2+} channels (Santicioli et al., 1992; Cahill et al., 1993) have been implicated in the release of neurotransmitters/neuromodulators from sensory neurons in the spinal cord. Ca^{2+} entry into dorsal root ganglion neurons also can occur via a cation channel that is activated by capsaicin (Holzer, 1991). Ruthenium red blocks the capsaicin-induced influx of Ca^{2+} into sensory neurons (Wood et al., 1988; Dray et al., 1990), as well as the capsaicin-induced release of neuropeptides (Maggi et al., 1988; Amann and Maggi, 1991; Donnerer et al., 1992) and adenosine (Cahill et al., 1993) from such neurons.

The release of adenosine by morphine contributes to spinal antinociception by morphine, as antinociception produced by intrathecal administration of morphine is reduced by methylxanthine adenosine receptor antagonists (DeLander and Hopkins, 1986; Sweeney et al., 1987). Although this release of adenosine is dependent on the external Ca^{2+} concentration (Sweeney et al., 1987), little is known of the mechanism by which Ca^{2+} enters the cell. In this study, we examine whether Ca^{2+} entry via L- or N-type Ca^{2+} channels, or the capsaicin-sensitive cation channel, is involved in the action of morphine by determining the effects of a dihydropyridine Ca^{2+} channel agonist and antagonist, ω -conotoxin, and ruthenium red on the morphine-evoked release of adenosine from spinal cord synaptosomes. As a prelude to these experiments, the effect on morphine of a partial depolarization with a minimally effective concentration of K^+ was examined to determine whether release of adenosine could occur at a lower concentration of morphine. The doses of morphine required to evoke adenosine release are relatively high (micromolar) compared with those shown in recent studies to modulate the release of substance P from an *in vitro* preparation (Maixner et al., 1990), and the partial depolarization with 6 mM K^+ reveals an effect of Bay K 8644 on adenosine release which is not seen under normal circumstances (Cahill et al., 1993).

MATERIALS AND METHODS

Release of adenosine from spinal cord synaptosomes

A crude P_2 synaptosomal fraction was prepared from the dorsal spinal cord of rats, as described previously (Cahill et al., 1993). The synaptosomal fraction was resuspended in Krebs-Henseleit medium to yield a final protein concentration of 2–3 mg/ml. This suspension (350 μl) was added to tubes containing the media and drugs (15 μl) and incubated for 15 min at 37°C. One tube was centrifuged immediately on addition of the synaptosomes to indicate the quantity of adenosine released during preparation of the synaptosomes (0 min value). The reaction was terminated by centrifugation followed by deproteination with ZnSO_4 and $\text{Ba}(\text{OH})_2$ and further centrifugation. The supernatant was derivatized by addition of 4.5% chloroacetaldehyde, and etheno-adenosine was quantitated by HPLC with fluorescence detection. Values are expressed as picomoles per milligram of protein per 15 min.

For Ca^{2+} -free experiments, the synaptosomes were prepared and resuspended in Ca^{2+} -free Krebs-Henseleit medium. During the incubations, 1 mM EGTA (buffered to pH 7.4 with 1 M NaOH) was included in the medium. Ca^{2+} -containing tubes had the normal 1.8 mM Ca^{2+} added back to the synaptosomes.

Drug treatments and expression of results

Basal adenosine values were calculated by subtracting release at 0 min from the release of adenosine in the absence of drugs. Evoked values were calculated by subtracting the total release in the presence of drugs from that in the absence of drugs. For Bay K 8644, nifedipine, ω -conotoxin, and ruthenium red experiments, release in the presence of these agents was subtracted from release in the presence of morphine and the agent generating the morphine-evoked release. This method potentially accounts for any intrinsic effects on basal release of adenosine produced by these agents. However, it should be noted that none of these treatments produced a significant alteration in the basal release of adenosine.

The dihydropyridines were dissolved in 25% dimethyl sulfoxide, which was diluted to 0.34% when added to synaptosomes. This concentration of solvent had no effect on basal adenosine release. ω -Conotoxin and ruthenium red were dissolved in saline. When examining effects of dihydropyridines, light restrictions were applied to minimize degradation.

Statistics

Data were analyzed using analysis of variance followed by the Student-Newman-Keuls test.

Drugs

Drugs were obtained from the following sources: Bay K 8644 (Miles, Inc., Pharmaceutical Division, Elkhart, IN, U.S.A.), nifedipine, ruthenium red, dimethyl sulfoxide, and EGTA (Sigma Chemical Co., St. Louis, MO, U.S.A.), ω -conotoxin (Peninsula Laboratories, Belmont, CA, U.S.A.), morphine sulphate (British Drug Houses, Ontario, Canada).

RESULTS

Characterization of morphine-evoked release of adenosine

Morphine (10^{-5} and 10^{-4} M) produced a dose-related increase in the release of adenosine from dorsal

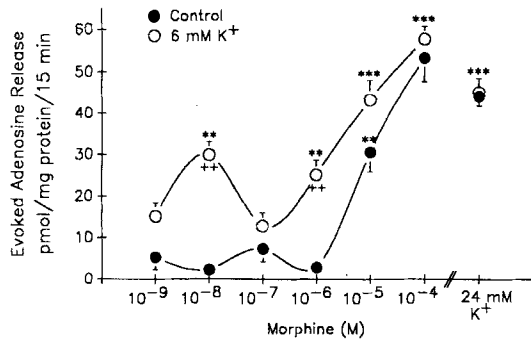


FIG. 1. Morphine-evoked release of adenosine from dorsal spinal cord synaptosomes in the absence (●) or presence (○) of 6 mM K^+ added to the normal Krebs–Henseleit medium. Basal adenosine release was 172 ± 7 pmol/mg of protein/15 min; 6 mM K^+ adenosine release was 180 ± 5 pmol/mg of protein/15 min. Values represent means \pm SEM. Where no error bars are indicated, SEM values are contained within the symbol. ** $p < 0.01$, *** $p < 0.001$, compared with basal release values; ++ $p < 0.01$, compared with release in the absence of the added 6 mM K^+ ($n = 6$).

spinal cord synaptosomes (Sweeney et al., 1987). No significant effect was seen between 10^{-9} and 10^{-6} M morphine (Fig. 1). The extent of the increase at 10^{-4} M morphine (40–50 pmol/mg of protein/15 min) (see also Figs. 3, 4, and 5) was similar to that produced by the addition of 24 mM K^+ (total K^+ concentration of 28.7 mM), a maximally effective concentration of K^+ . It is interesting that following the addition of 6 mM K^+ (total K^+ concentration of 10.7 mM), which by itself did not significantly alter the release of adenosine, a significant enhancement of release occurred at 10^{-8} and 10^{-6} M morphine but not at 10^{-7} M morphine (Fig. 1). The enhancement of adenosine release seen at 10^{-8} M morphine (30 pmol/mg of protein/15 min) was most consistently seen in the presence of the additional 6 mM K^+ (Figs. 3C and 4B, but see Fig. 2). In some instances, there appeared to be some stimula-

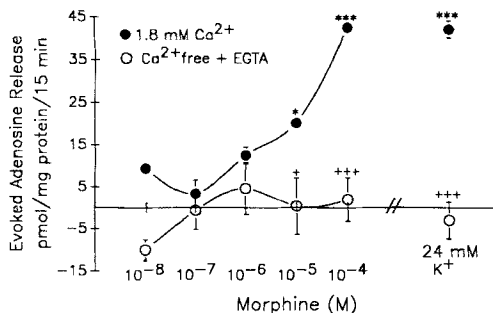


FIG. 2. Calcium-dependent release of adenosine produced by morphine in the presence of an additional 6 mM K^+ . Basal adenosine release was 178 ± 5 pmol/mg of protein/15 min; basal Ca^{2+} -free/EGTA adenosine release was 220 ± 9 pmol/mg of protein/15 min ($p < 0.01$, compared with basal value). * $p < 0.05$, *** $p < 0.001$, compared with basal release; + $p < 0.05$, +++ $p < 0.001$, compared with release in the presence of Ca^{2+} ($n = 6$).

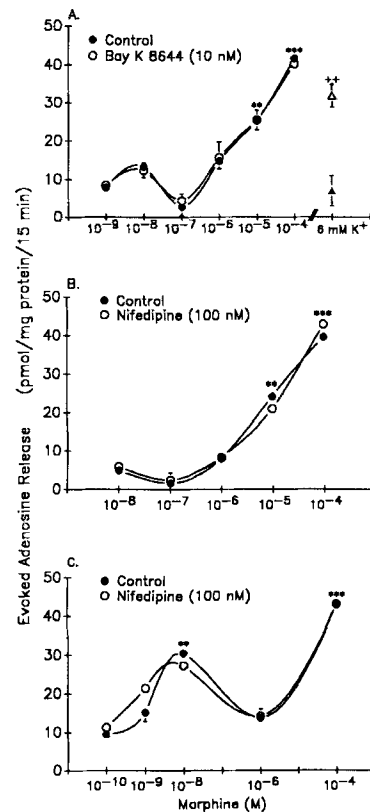


FIG. 3. Effects of 10 nM Bay K 8644 (A) and 100 nM nifedipine (B and C) on the morphine-evoked release of adenosine in the absence (A, B) or presence (C) of an additional 6 mM K^+ . Mean basal adenosine release values ranged from 182 to 209 pmol/mg of protein/15 min. Neither the solvent, Bay K 8644, nor nifedipine treatments had any significant effect on basal release values. ** $p < 0.01$, *** $p < 0.001$, compared with basal values; ++ $p < 0.01$, compared with release in the presence of the added 6 mM K^+ ($n = 4$ in all cases).

tion of release by 10^{-8} M morphine in the absence of added K^+ (Figs. 3A and 4A), but this effect was < 20 pmol/mg of protein/15 min, and did not attain statistical significance.

The enhanced release of adenosine by morphine following the addition of 6 mM K^+ was Ca^{2+} dependent, as no significant alteration in release was seen in the absence of Ca^{2+} and presence of 1 mM EGTA to chelate residual Ca^{2+} (Fig. 2). Previously, morphine-evoked release of adenosine in the absence of the added K^+ was shown to be Ca^{2+} dependent (Sweeney et al., 1987). The K^+ -evoked release of adenosine (by addition of 24 mM K^+) also was Ca^{2+} dependent (Fig. 2; Sweeney et al., 1987). It should be noted that the Ca^{2+} -free condition enhanced the basal release of adenosine (see legend to Fig. 2), but unlike morphine- and K^+ -evoked release of adenosine (Sweeney et al., 1987), much of this originated as nucleotide rather than as adenosine (C. M. Cahill, T. D. White, and J. Sawynok, unpublished observations).

Effects of dihydropyridines, ω -conotoxin, and ruthenium red on morphine-evoked release of adenosine

Bay K 8644 (10 nM), at a dose previously shown to maximally augment adenosine release evoked by a partially effective concentration of K^+ (EC_{50} value approximately 2 nM; Cahill et al., 1993), had no significant effect on release of adenosine evoked by morphine at any dose (Fig. 3A). This dose significantly enhanced the release of adenosine by addition of 6 mM K^+ , a condition included in the same experiment as a positive control (Fig. 3A). Similarly, 100 nM nifedipine, a dose previously shown to maximally inhibit the augmented release of adenosine by Bay K 8644/6 mM K^+ (IC_{50} value approximately 0.1 nM; Cahill et al., 1993), had no effect on release of adenosine evoked by morphine either in the absence (Fig. 3B) or in the presence of the additional 6 mM K^+ (Fig. 3C).

In contrast to the lack of effect with dihydropyridines, 100 nM ω -conotoxin, a dose previously shown to inhibit K^+ -evoked release of adenosine (IC_{50} value of 8 nM; Cahill et al., 1993), inhibited the release of

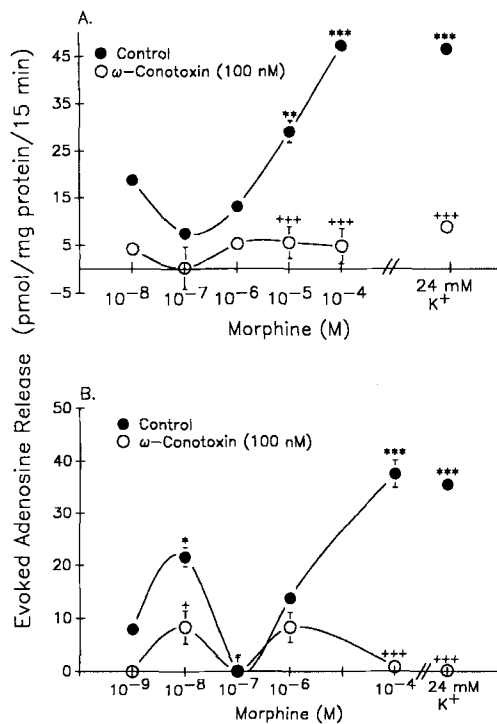


FIG. 4. Inhibition of morphine-evoked release of adenosine by ω -conotoxin in the absence (A) and presence (B) of an additional 6 mM K^+ . In A, basal adenosine release was 200 ± 4 pmol/mg of protein/15 min in the absence and 202 ± 8 pmol/mg of protein/15 min in the presence of ω -conotoxin. In B, basal adenosine release was 193 ± 8 pmol/mg of protein/15 min in the absence and 192 ± 2 pmol/mg of protein/15 min in the presence of ω -conotoxin. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared with basal values; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared with release evoked by morphine (A) or by morphine/6 mM K^+ (B). $n = 6$.

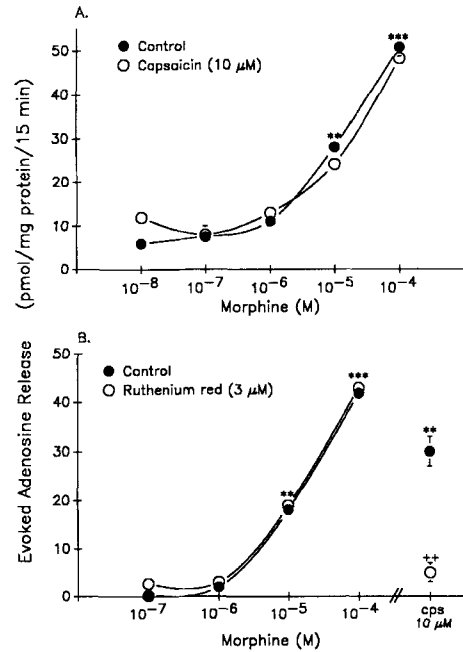


FIG. 5. Lack of effect of 10 μ M capsaicin (A) and 3 μ M ruthenium red (B) on morphine-evoked release of adenosine. In A, basal adenosine release was 135 ± 5 pmol/mg of protein/15 min in the absence and 155 ± 7 pmol/mg of protein/15 min in the presence of capsaicin. In B, basal adenosine release was 142 ± 6 pmol/mg of protein/15 min in the absence and 145 ± 5 pmol/mg of protein/15 min in the presence of ruthenium red. ** $p < 0.01$, *** $p < 0.001$, compared with basal release; ++ $p < 0.01$, compared with release evoked by capsaicin (cps). $n = 4$.

adenosine induced by morphine alone (10^{-5} and 10^{-4} M, Fig. 4A) as well as the additional component of release evoked by 10^{-8} M morphine in the presence of the additional 6 mM K^+ (Fig. 4B). This dose of ω -conotoxin consistently inhibited release of adenosine evoked by addition of 24 mM K^+ in the same experiment (Fig. 4A and B).

Capsaicin (1–100 μ M) induces a dose-dependent increase in release of adenosine from dorsal spinal cord synaptosomes (Sweeney et al., 1989). A partially effective dose of capsaicin did not alter release of adenosine evoked by a range of morphine concentrations (Fig. 5A). Ruthenium red (3 μ M), which markedly attenuates capsaicin-evoked release of adenosine (IC_{50} value of 1 μ M; Cahill et al., 1993, Fig. 5B), also did not alter release of adenosine evoked by morphine (Fig. 5B).

Effects of dihydropyridines, ω -conotoxin, and ruthenium red on basal release of adenosine

None of the agents that modulate Ca^{2+} entry examined here had any significant effect on the basal release of adenosine (see figure legends, and Cahill et al., 1993). This lack of effect is consistent with the observation that in the absence of a depolarizing stimulus, nifedipine and ω -conotoxin have minimal effects on Ca^{2+} uptake into synaptosomes (Adamson et al., 1989).

DISCUSSION

The present study demonstrates that there are two distinct phases (nanomolar and micromolar) in the morphine-evoked release of adenosine from dorsal spinal cord synaptosomes. The component of release that occurs at nanomolar concentrations is observed most consistently when the synaptosomes are partially depolarized by the addition of a minimally effective concentration of K^+ (6 mM; total K^+ concentration of 10.7 mM). Previously, micromolar concentrations of morphine had been shown to enhance adenosine release from spinal cord synaptosomes (Sweeney et al., 1987). This action was considered relevant to spinal antinociception in view of observations in behavioral experiments that methylxanthine adenosine receptor antagonists reduced spinal antinociception by morphine (DeLander and Hopkins, 1986; Sweeney et al., 1987). Although the dose of morphine required to produce this effect was relatively high compared with affinities of morphine at opioid receptors (K_D and IC_{50} values in the nanomolar range; e.g., Pasternak and Wood, 1986), it was comparable to doses required to inhibit the release of substance P from *in vitro* preparations (Jessell and Iversen, 1977; Pang and Vasko, 1986). More recent studies have demonstrated that substance P release can be inhibited by much lower concentrations of morphine (Maixner et al., 1990). In the present study, the unmasking of a potent (nanomolar) stimulating effect of morphine in the presence of 6 mM K^+ indicates that an enhanced release of adenosine can occur at doses that are comparable to those effective at displacing ligands in binding studies. It is interesting that this dose also is comparable to the nanomolar range of doses of opioids that have been shown to prolong the action potential duration in cultured sensory neurons (Crain and Shen, 1990). This observation thus provides more convincing evidence that opioid-induced release of adenosine may be relevant to antinociception, at least when afferent nerve terminals are partially depolarized.

The second major observation of this study is that both phases of adenosine release induced by morphine are dependent on the external Ca^{2+} concentration, and appear to result from Ca^{2+} entry into the synaptosome via an ω -conotoxin-sensitive N-type Ca^{2+} channel. Reports that morphine can directly enhance Ca^{2+} entry into neurons and neuroblastoma or neuroblastoma hybrid cells (Barr and Leslie, 1985; Lorentz et al., 1988; Jin et al., 1991) are consistent with the notion of an enhanced Ca^{2+} entry contributing to this action. Although sensory neurons contain functionally active L-type Ca^{2+} channels (Perney et al., 1986; Holz et al., 1988) and ruthenium red-sensitive Ca^{2+} uptake sites (Wood et al., 1988; Dray et al., 1990; Donnerer et al., 1992) that regulate the release of neuropeptides, L-type and ruthenium red-sensitive cation channels do not appear to be involved in the

morphine-induced release of adenosine as neither nifedipine nor ruthenium red affected the morphine-evoked release of adenosine. Although some studies have suggested that ω -conotoxin acts at both N-type and L-type Ca^{2+} channels (McCleskey et al., 1987; Holz et al., 1988), the effect of ω -conotoxin here is not mimicked by the dihydropyridine L-type Ca^{2+} channel antagonist nifedipine. In addition, other studies have demonstrated that ω -conotoxin and dihydropyridine binding sites differ in absolute numbers and regional distributions (Dooley et al., 1988; Kerr et al., 1988; Wagner et al., 1988), and that there is no competition between the binding of ω -conotoxin and drugs that act at the L-type Ca^{2+} channel (Cruz and Olivera, 1986), indicating a distinct site of action of ω -conotoxin and dihydropyridines.

ω -Conotoxin has been shown to block both the K^+ -stimulated uptake of Ca^{2+} into synaptosomes (Reynolds et al., 1986; Rivier et al., 1987), and the K^+ -evoked release of adenosine from spinal cord synaptosomes (Cahill et al., 1993; Fig. 4A and B). Morphine has been reported to produce a direct depolarization of neuroblastoma and neuroblastoma hybrid cells (Lorentz et al., 1988). Morphine does not appear to release adenosine from spinal cord synaptosomes by depolarizing synaptosomal membranes in a manner identical to K^+ , as Bay K 8644 modulates the release of adenosine produced by partial depolarization with submaximally effective K^+ concentrations (Cahill et al., 1993) but not submaximally effective morphine concentrations (Fig. 3A). There is a brief report that opioids can stimulate Ca^{2+} entry into neuroblastoma hybrid cells by activating a protein kinase-sensitive Na^+ conductance that leads to the recruitment of voltage-sensitive Ca^{2+} channels that are blocked by dihydropyridines (Jin et al., 1991). This specific mechanism does not appear to be the basis of the morphine-induced increase in adenosine release, as dihydropyridine-sensitive Ca^{2+} channels are not involved in the morphine-evoked release of adenosine. There does however remain the possibility that this type of stimulatory effect of opioids on Ca^{2+} influx in different cell types is mediated by different types of Ca^{2+} channels, as suggested by our findings with ω -conotoxin. It will also be important to resolve the opioid receptor subtypes that promote Ca^{2+} entry, as different subtypes may interface with different Ca^{2+} channels.

Opioids inhibit the release of a variety of neurotransmitters and neuromodulators from a variety of locations in the peripheral and central nervous systems (Illes, 1989), as well as in the spinal cord (Jessell and Iversen, 1977; Yaksh et al., 1980; Lembeck and Donnerer, 1985; Pang and Vasko, 1986; Aimone and Yaksh, 1989; Pohl et al., 1989). This inhibition is thought to be due to inhibition of Ca^{2+} uptake into nerve terminals and inhibition of the action potential duration (see introductory section for references). Stimulatory effects of opioids on Ca^{2+} uptake (Barr

and Leslie, 1985; Lorentz et al., 1988; Jin et al., 1991) and an enhanced action potential duration (Crain and Shen, 1990) have received attention only recently. Concurrently, opioid effects on neurotransmitter release are becoming more complex. Thus, there are reports of stimulation of the release of substance P from the spinal cord and trigeminal nucleus (Mauborgne et al., 1987; Maixner et al., 1990; Suárez-Roca et al., 1990; Suárez-Roca and Maixner, 1991), of cholecystinin from the spinal cord (Benoliel et al., 1991), and of methionine-enkephalin from the myenteric plexus (Xu et al., 1989) by certain doses of morphine or ligands selective for opioid receptor subtypes. It is interesting that the enhancement of methionine-enkephalin release from the myenteric plexus by low concentrations of opioids appears to be mediated by an increase in cyclic AMP (Xu et al., 1989; Gintzler and Xu, 1991). This finding corresponds well with electrophysiological data indicating that an increase in cyclic AMP is involved in the prolongation of action potential duration seen in cultured sensory neurons (Chen et al., 1988; Shen and Crain, 1989; 1990; Crain and Shen, 1990). Although an enhancement of cyclic AMP does not appear to mediate the morphine-evoked release of adenosine at micromolar concentrations (Nicholson et al., 1991), it would be interesting to determine whether the nanomolar phase of adenosine release by morphine is regulated by this intracellular mechanism.

Ca²⁺ entry into a variety of cell types is regulated by G proteins (Schultz et al., 1990; Dolphin, 1990). Pertussis toxin-sensitive G proteins (e.g., G_i family and G_o) mediate agonist-induced (including opioid-induced) inhibition of Ca²⁺ currents in neurons and neuroblastoma hybrid cells (Hescheler et al., 1987; Toselli and Lux, 1989). Pertussis toxin-sensitive inhibitory regulation of Ca²⁺ entry into neurons occurs via N-type Ca²⁺ channels (Dolphin, 1990). In other cell types (endocrine cells, cardiac and skeletal muscle myocytes), G proteins are involved in stimulation of Ca²⁺ entry into the cell (Schultz et al., 1990). It is surprising that stimulation of Ca²⁺ currents also is sensitive to pertussis toxin (Kojima et al., 1986; Hescheler et al., 1988; Rosenthal et al., 1988). It is also interesting to note that the morphine-evoked release of adenosine both in vitro and in vivo is sensitive to pertussis toxin (Sawynok et al., 1990), suggesting that opioid-induced stimulation of Ca²⁺ entry into neurons may also involve a G protein.

In summary, the present study demonstrates that morphine can produce a biphasic enhancement (nanomolar and micromolar) of adenosine release from dorsal spinal cord synaptosomes that have been partially depolarized with K⁺. The nanomolar component of action corresponds well with doses required to enhance action potential duration in cultured sensory neurons. Both phases of release are due to Ca²⁺ entry into the synaptosome by ω-conotoxin-sensitive N-

type Ca²⁺ channels. L-type Ca²⁺ channels and ruthenium red-sensitive Ca²⁺ entry do not appear to be involved in this action.

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