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Short communication

Substance P releases and augments the morphine-evoked release of adenosine from spinal cord

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Abstract

The effects of substance P on the morphine-evoked release of adenosine were examined. Substance P alone produced a multiphasic effect on release of adenosine, with release occurring at low nanomolar concentrations and at a micromolar concentration, but not at intermediate concentrations. An inactive dose of substance P augmented the morphine-evoked release of adenosine at a nanomolar concentration of morphine. Release of adenosine by substance P alone (1 nM) or substance P/morphine (100 nM/10 nM) was Ca^{2+} -dependent and originated from capsaicin-sensitive nerve terminals. © 1997 Elsevier Science B.V.

Keywords: Adenosine; Substance P; Morphine; Spinal cord

Substance P is present in small diameter unmyelinated primary afferent nerve terminals within the dorsal spinal cord and is involved in the transmission/modulation of nociceptive information [15]. Substance P depolarizes projection neurons and interneurons within the dorsal horn, and such postsynaptic actions have received emphasis with respect to pain transmission mechanisms [15]. There is also some evidence that substance P can modulate primary afferent function [10,17]. Substance P is released from primary afferent neurons by noxious stimulation [15] and release is increased under conditions of inflammation [6,19]. Opioids have been known for some time to inhibit the release of substance P from sensory nerve terminals contributing to antinociception [9], but more recent studies report dual effects of opioids on substance P release with stimulatory and inhibitory effects being due to actions on different opioid receptor populations [21]. At supraspinal sites, substance P releases endogenous opioids [8,14], and this contributes to some behavioural effects of substance P. Multiple forms of interactions appear to occur between opioids and substance P in relation to pain mechanisms.

Within the spinal cord, release of adenosine mediates a component of morphine-induced antinociception. In behavioural studies, spinal opioid-induced antinociception is antagonized by pretreatment with methylxanthines [2,4], while in neurochemical studies, opioids stimulate the release of adenosine in both in vivo and in vitro spinal cord preparations [22]. The morphine-evoked release of adenosine from dorsal spinal cord synaptosomes occurs at nanomolar concentrations in the presence of elevated K^+ concentrations; this release occurs via activation of μ -opioid receptors [2]. The present study determined whether substance P can induce adenosine release directly, and whether it augments morphine-evoked release of adenosine from dorsal spinal cord synaptosomes in a manner similar to K^+ .

Male Sprague–Dawley rats (250–325 g; Charles River, Quebec, Canada) were used. Adenosine release from dorsal spinal cord synaptosomes was examined in a synaptosomal suspension as described previously in detail [2]. For intrathecal pretreatment with capsaicin, an acute cannula was inserted into the spinal subarachnoid space under halothane anaesthesia as described previously [22]. Capsaicin (60 μg in 20 μl 60% dimethylsulfoxide/saline) or vehicle was injected over a 7–10-min interval prior to cannula withdrawal. Animals were allowed to recover at least 7 days before being used in neurochemical experiments. Any animal displaying motor deficits as a result of this procedure was excluded. For Ca^{2+} -free experiments,

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synaptosomes were prepared in a Krebs-Henseleit medium from which Ca^{2+} was omitted. Ca^{2+} was added back to synaptosomes during the incubation stage. All experiments included a time = 0 determination of adenosine generated by the experimental procedure, and this was subsequently subtracted from all other values. Adenosine release values are expressed as pmol adenosine released per mg protein. Statistical comparisons were made using analysis of variance and Student Newman Keuls test for post hoc comparisons.

Substance P released adenosine in a multiphasic manner, enhancing release at 0.1–1 nM, and again at 1 μM but not at intermediate concentrations (Fig. 1A). The extent of the adenosine released by substance P at both concentrations is comparable to that produced by maximum depolarization with K^+ (cf. [3]). Two threshold concentrations of substance P (0.01 nM and 100 nM) were combined with morphine. Substance P at 100 nM enhanced release of adenosine by 10 nM morphine (Fig. 1B), as does 6 mM K^+ (cf. [3]). No augmentation of release was observed with 0.01 nM substance P (data not shown). The release of adenosine evoked by substance P (1 nM) and substance P/morphine (100 nM/10 nM) appears to originate from capsaicin-sensitive nerve terminals, as release from capsaicin-pretreated rats was significantly reduced (Fig. 2A). Such release was Ca^{2+} -dependent, as no release occurred when Ca^{2+} was omitted from the medium (Fig. 2B). These characteristics of release are identical to those observed for morphine in the presence of 6 mM K^+ (Fig. 2A,B).

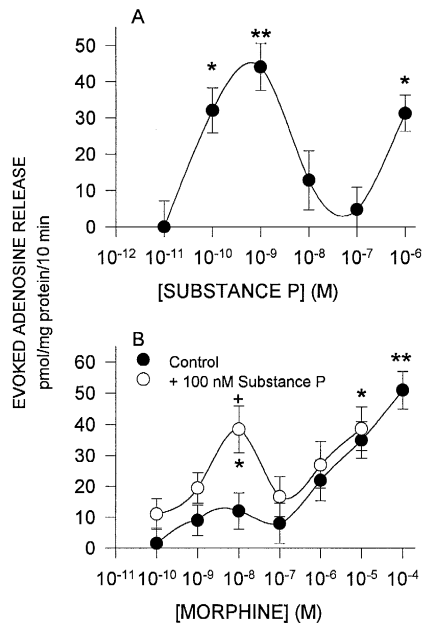


Fig. 1. A: dose-related release of adenosine by substance P from dorsal spinal cord synaptosomes. B: substance P enhances morphine-evoked release of adenosine. Values represent mean \pm S.E.M. for $n = 5$. * $P < 0.05$, ** $P < 0.01$ compared to basal, + $P < 0.05$ compared to release in absence of substance P. Basal adenosine release values ranged from 190 to 245 ± 17 pmol/mg protein/10 min.

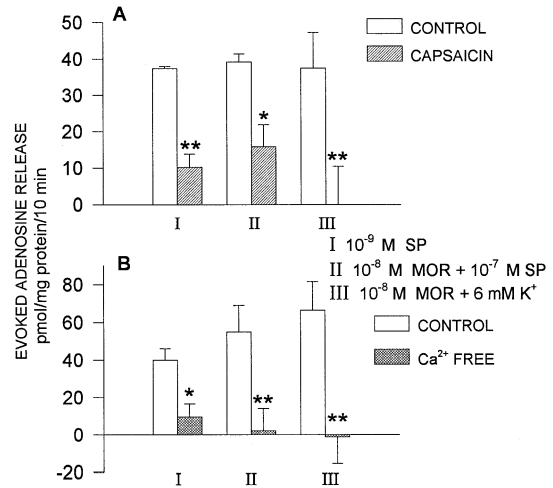


Fig. 2. Capsaicin sensitivity (A) and calcium dependency (B) of evoked adenosine release by substance P, morphine/substance P, and morphine/ K^+ . Values represent mean \pm S.E.M. for $n = 5$. Panel A: * $P < 0.05$, ** $P < 0.01$ compared to evoked release from synaptosomes prepared from vehicle treated animals. Panel B: * $P < 0.05$, ** $P < 0.01$ compared to evoked release under normal Ca^{2+} concentrations (1.8 mM). Basal values ranged from 200 to 245 ± 15 pmol/mg protein/10 min.

The present study demonstrates that substance P can release adenosine from dorsal spinal cord synaptosomes in a Ca^{2+} -dependent manner. Substance P depolarizes a range of neuronal types by decreasing K^+ conductances, leads to enhanced Ca^{2+} entry via voltage-gated Ca^{2+} channels, and induces Ca^{2+} release from intracellular stores [15]. Substance P releases a number of neurotransmitters from spinal cord preparations; in some cases release is Ca^{2+} -dependent [11], but in others, it is Ca^{2+} -independent [10,18], perhaps reflecting an involvement of different neurokinin receptors in these responses. An interesting feature of the adenosine release induced by substance P is its multiphasic nature. The neurokinin receptor subtype mediating release of adenosine by substance P at nanomolar concentrations is likely a neurokinin-1 receptor based on the potency of the effect [15]; other subtypes may mediate the inhibitory phase and subsequent stimulatory phase at higher concentrations. Micromolar concentrations of substance P previously have been shown to release glutamate, acetylcholine and gamma-aminobutyric acid from spinal cord preparations [10,11,18].

The capsaicin-sensitivity of the substance P-induced release of adenosine suggests that release occurs from small diameter primary afferent nerve terminals, as the capsaicin pretreatment schedule used here results in degeneration of C fibre profiles in the substantia gelatinosa [16]. A number of observations suggest that substance P can exert actions on afferent nerve terminals within the spinal cord. Thus, substance P releases glutamate from primary afferents [10], alters primary afferent nerve terminal excitability [17], and depolarizes sensory neuron cell bodies [20]. Ligand binding studies have failed to demonstrate any

loss of substance P receptors in the dorsal horn following capsaicin pretreatment or rhizotomy [13,25], but post-synaptic upregulation may have obscured a change in a small population of receptors. More recently, *in situ* hybridization analysis of mRNA for substance P receptors and immunohistochemistry of the substance P receptor itself in the spinal cord showed no evidence of substance P receptors on primary afferent nerve terminals [1], and it was suggested that effects of substance P on C fibres are mediated indirectly by actions on interneurons. In the present study, release occurs from a synaptosomal suspension where anatomical juxtapositions are largely not retained. This observation initially suggests that a direct effect on synaptosomes occurs, perhaps by a direct depolarization. However, an indirect effect via release of endogenous opioids also is possible. Thus, spinal administration of substance P can produce a delayed analgesia which is blocked both by naloxone (suggesting release of endogenous opioids) [5,23], and by caffeine (suggesting an adenosine link also occurs) [24]. The present demonstration that the effect of a nanomolar concentration of morphine is enhanced by substance P indicates that an amplification mechanism could occur in the synaptosomal suspension due to simple diffusion of a mediator without necessarily requiring an anatomical juxtaposition. Opioid-induced release of adenosine is capsaicin-sensitive [22], and this would then account for the capsaicin-sensitivity of the adenosine released by substance P and the substance P/morphine combination.

The interaction between substance P and morphine in releasing adenosine is of interest from a functional point of view. Substance P is released by acute noxious sensory stimulation [15], and this could interact subsequently with morphine to augment antinociception. The spinal administration of low doses of substance P has been shown to potentiate antinociception by morphine using the thermal threshold tail flick test, and this exhibits a bell-shaped dose-response curve as does adenosine release [12]. Augmentation of the action of morphine could occur either by substance P releasing adenosine directly with adenosine subsequently enhancing the action of morphine [3], or substance P enhancing the ability of morphine to release adenosine and accentuating the component of opioid action due to adenosine release [2,4]. Interestingly, under conditions of inflammation where release of substance P is enhanced [6,19], morphine exhibits an enhanced spinal antinociception [7]. A substance P-adenosine-opioid axis could contribute to changes which occur under conditions of inflammation as well.

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References

- [1] J.L. Brown, H. Liu, J.E. Maggio, S.R. Vigna, P.W. Mantyh, A.I. Basbaum, Morphological characterization of substance P receptor-immunoreactive neurons in the rat spinal cord and trigeminal nucleus caudalis, *J. Comp. Neurol.* 356 (1995) 327–344.
- [2] C.M. Cahill, T.D. White, J. Sawynok, Spinal opioid receptors and adenosine release: neurochemical and behavioral characterization of subtypes, *J. Pharmacol. Exp. Ther.* 275 (1995) 84–93.
- [3] G.E. DeLander, G.J. Keil, Antinociception induced by intrathecal coadministration of selective adenosine receptor and selective opioid receptor agonists in mice, *J. Pharmacol. Exp. Ther.* 268 (1994) 943–951.
- [4] G.E. DeLander, H.I. Mosberg, F. Porreca, 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 (1992) 1097–1104.
- [5] T. Doi, I. Jurna, Intrathecal substance P depresses the tail flick response — antagonism by naloxone, *Naunyn-Schmiedeberg's Arch. Pharmacol.* 317 (1981) 135–139.
- [6] M.G. Garry, K.M. Hargreaves, Enhanced release of immunoreactive CGRP and substance P from spinal dorsal horn slices occurs during carrageenan inflammation, *Brain Res.* 582 (1992) 139–142.
- [7] J.L.K. Hylden, D.A. Thomas, M.J. Iadarola, R.L. Nahin, R. Dubner, Spinal opioid analgesic effects are enhanced in a model of unilateral inflammation/hyperalgesia: possible involvement of noradrenergic mechanisms, *Eur. J. Pharmacol.* 194 (1991) 135–143.
- [8] M.J. Iadarola, J. Tang, E. Costa, H.-Y.T. Yang, Analgesic activity and release of [Met⁵]Enkephalin-Arg⁶-Gly⁷-Leu⁶ from rat spinal cord *in vivo*, *Eur. J. Pharmacol.* 121 (1986) 39–48.
- [9] T.M. Jessell, L.L. Iversen, Opiate analgesics inhibit substance P release from rat trigeminal nucleus, *Nature* 268 (1977) 549–551.
- [10] I. Kangrga, M. Randic, Tachykinins and calcitonin gene-related peptide enhance release of endogenous glutamate and aspartate from the rat spinal dorsal horn slice, *J. Neurosci.* 10 (1990) 2026–2038.
- [11] N. Kobayashi, M. Sakuma, K. Yoshioka, Y. Onishi, M. Yanagisawa, K. Kawashima, M. Otsuka, Substance P-evoked release of acetylcholine from isolated spinal cord of the newborn rat, *Neuroscience* 45 (1991) 331–337.
- [12] R.M. Cream, T. Kato, H. Shimonaka, J.E. Marchand, W.H. Wurm, Substance P markedly potentiates the antinociceptive effects of morphine sulfate administered at the spinal level, *Proc. Natl. Acad. Sci. USA* 90 (1993) 3564–3568.
- [13] P.W. Mantyh, S.P. Hunt, The autoradiographic localization of substance P receptors in the rat and bovine spinal cord and the rat and cat spinal trigeminal nucleus pars caudalis and the effects of neonatal capsaicin, *Brain Res.* 332 (1985) 315–324.
- [14] J.R. Naranjo, A. Arnedo, M.C. De Felipe, J. Del Rio, Antinociceptive and Met-enkephalin releasing effects of tachykinins and substance P fragments, *Peptides* 7 (1986) 419–423.
- [15] M. Otsuka, K. Yoshioka, Neurotransmitter functions of mammalian tachykinins, *Physiol. Rev.* 73 (1993) 229–308.
- [16] N.N. Palermo, H.K. Brown, D.L. Smith, Selective neurotoxic action of capsaicin on glomerular C-type terminals in rat substantia gelatinosa, *Brain Res.* 208 (1981) 506–510.
- [17] M. Randic, E. Carstens, M. Zimmermann, D. Klumpp, Dual effects of substance P on the excitability of single cutaneous primary afferent C- and A-fibers in the cat spinal cord, *Brain Res.* 233 (1982) 389–393.
- [18] M. Sakuma, K. Yoshioka, H. Suzuki, M. Yanagisawa, Y. Onishi, N. Kobayashi, M. Otsuka, Substance P-evoked release of GABA from isolated spinal cord of the newborn rat, *Neuroscience* 45 (1991) 323–331.
- [19] H.-G. Schaible, B. Jarrott, P.J. Hope, A.W. Duggan, Release of immunoreactive substance P in the spinal cord during development of acute arthritis in the knee joint of the cat: a study with antibody microprobes, *Brain Res.* 529 (1990) 214–223.

- [20] I. Spigelman, R.L. Puil, Substance P actions on sensory neurons, *Ann. NY Acad. Sci.* 632 (1991) 220–228.
- [21] H. Suarez-Roca, W. Maixner, Morphine produces a multiphasic effect on the release of substance P from rat trigeminal nucleus slices by activating different opioid receptor subtypes, *Brain Res.* 579 (1992) 195–203.
- [22] M.I. Sweeney, T.D. White, J. Sawynok, Morphine, capsaicin and K^+ release purines from capsaicin-sensitive primary afferent nerve terminals in the spinal cord, *J. Pharmacol. Exp. Ther.* 248 (1989) 447–454.
- [23] K. Yashpal, J.L. Henry, Endorphins mediate overshoot of substance P-induced facilitation of a spinal nociceptive reflex, *Can. J. Physiol. Pharmacol.* 61 (1982) 303–307.
- [24] K. Yashpal, J.L. Henry, Adenosine receptor link in an adrenal opioid-induced antinociception in the rat tail-flick test, *Neurosci. Lett.* 138 (1992) 253–256.
- [25] K. Yashpal, T.-V. Dam, R. Quirion, Effects of dorsal rhizotomy on neurokinin receptor subtypes in the rat spinal cord: a quantitative autoradiographic study, *Brain Res.* 552 (1991) 240–247.