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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 \(\mu\)-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 synapticomal 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 \(\mu\)g in 20 \(\mu\)l 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,
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 Ca2+-dependent, as no release occurred when Ca2+ 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).

The present study demonstrates that substance P can release adenosine from dorsal spinal cord synaptosomes in a Ca2+-dependent manner. Substance P depolarizes a range of neuronal types by decreasing K+ conductances, leads to enhanced Ca2+ entry via voltage-gated Ca2+ channels, and induces Ca2+ release from intracellular stores [15]. Substance P releases a number of neurotransmitters from spinal cord preparations; in some cases release is Ca2+-dependent [11], but in others, it is Ca2+-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 postsynaptic 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
