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SPECIAL REPORT

NMDA receptor activation modulates evoked release of substance P from rat spinal cord

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The possible modulation exerted by glutamate on substance P (SP) release from the rat spinal cord has been investigated. The N-methyl-D-aspartate (NMDA) receptor agonist, NMDA (1 μM), increased SP basal outflow by 46.5±10.9% ($n=3$, $P<0.01$) without changing the evoked release of the peptide. Conversely, NMDA antagonists but not 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) inhibited both electrically-evoked and capsaicin-induced release of SP. In particular, D-2-amino-5-phosphonopentanoate (D-AP5; 50 μM) inhibited electrically-evoked and capsaicin-induced release of SP by 93±2.4% and 93.2±3.8% ($n=12$, $P<0.01$), respectively. Functional pharmacological evidence is provided for glutamate exerting a positive feedback on SP release evoked by C fibre stimulation *via* NMDA receptor activation.

Keywords: SP; glutamate; NMDA receptor; heteroreceptors; release; spinal cord; capsaicin; electrical stimulation

Introduction Substance P (SP) and glutamate which are contained in some C fibre terminals within the dorsal horn can interact at both the pre- and post-synaptic level. Released SP, whilst exerting a negative feedback on its own release *via* activation of NK_1 autoreceptors (Malcangio & Bowery, 1994), causes release of glutamate (Kangrga & Randic, 1990). On the other hand, glutamate can induce NK_1 receptor internalization (which is an indication that SP release has occurred; Mantyh *et al*., 1995), *via* activation of pre-synaptically located NMDA receptors (Liu *et al*., 1994, 1997). Furthermore, NMDA receptor antagonists selectively prevent NK_1 receptor internalization in the dorsal horn of the spinal cord induced by electrical stimulation of the dorsal roots (Marvizon *et al*., 1997). In the present study, we investigated whether agonists and/or antagonists for NMDA receptors could modulate the release of SP from the rat isolated spinal cord.

Methods Lumbo-sacral hemisected spinal cord slices (350 μm thick) with attached dorsal roots were obtained from adult rats and mounted in a three-compartment bath (Malcangio & Bowery, 1994). Each slice was continuously superfused at 1 ml min^{-1} at room temperature with oxygenated (5% CO_2) Krebs' solution (in mM): NaCl, 118; KCl, 4; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25; $CaCl_2$, 2.5 and glucose, 11, containing 0.1% bovine serum albumin, protease inhibitors (captopril 100 μM, phosphoramidon 1 μM, bacitracin 20 μg ml^{-1}) and dithiothreitol (6 μM). The dorsal roots were draped on electrodes into lateral compartments. Superfusates were collected for 8 min in acetic acid (0.1 M) in the following order: two fractions to measure basal outflow of SP, one fraction in the absence (control) or presence of drugs to measure effects on basal outflow, one fraction in the presence or absence (control) of drugs to either measure electrically-evoked (20 V, 0.5 ms at 1 Hz for 8 min, 12.5±1.2 mA) or capsaicin (10 nM for 2 min)-induced release of SP and three fractions to measure the return to basal levels. At the end of experiments, samples were desalted by using Sep-Pak C_{18} cartridges (Waters, Watford, U.K.) and SP-like immunoreactivity (SP-LI) content was determined by radioimmunoassay (Malcangio & Bowery, 1994).

Drugs used: N-methyl-D-aspartic acid and capsaicin (8-methyl-N-vanillyl-6-nonenamide) (SIGMA, U.K.); D(−)-2-amino-5-phosphonopentanoate (D-AP5) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (Research Biochemicals Internationals (RBI), U.S.A.).

Results Significant release of SP-LI from rat spinal cord slices was evoked by both electrical stimulation of the dorsal roots and superfusion with capsaicin. In both situations, SP-LI content, which at a basal level was 10.9±1.8 fmol 8 ml-fraction^{-1} ($n=12$), approximately doubled (22.3±1.3 and 19.0±1.2 fmol 8 ml-fraction^{-1} after electrical stimulation ($n=6$, $P<0.01$) and capsaicin superfusion ($n=6$, $P<0.01$), respectively. Slices superfused with the NMDA receptor agonist, NMDA (1 μM), increased SP-LI basal outflow (Table 1) without changing evoked release of the peptide (Table 1). Highly significant inhibition of evoked, but not basal release of SP-LI was observed when the selective NMDA antagonist, D-AP5 (50 μM), was superfused (Table 1). No changes in release of SP-LI were detected after superfusion with CNQX (5 μM).

Previous superfusion of spinal cord slices with D-AP5 (50 μM), but not CNQX (5 μM), significantly inhibited the release of SP-LI evoked by capsaicin (Figure 1). However, the presence of NMDA did not change the effect of capsaicin (Figure 1).

Discussion This study shows that a selective block of the NMDA receptor but not the CNQX-sensitive receptor in the dorsal horn of the spinal cord inhibits SP release induced by either electrical stimulation of the dorsal roots or capsaicin superfusion. In this study, the source of SP was likely to be mainly from the unmyelinated fibre terminals since capsaicin is a selective activator of C fibres and the electrically-evoked

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Table 1 Effect of NMDA and antagonist drugs on basal outflow and electrically-evoked SP-LI release from the rat spinal cord

	Substance P-LI (fmol 8 ml fraction^{-1})		
Drug	*Basal outflow in absence of drug*	*Changes in basal outflow in presence of drug*	*Electrically-evoked release in presence of drug*
None ($n=9$)	10.4±2.4	0.9±0.5	7.8±1.1
NMDA 1 μM	14.7±4.8 ($n=6$)	6.9±1.5* ($n=3$)	8.8±1.7 ($n=6$)
NMDA 10 μM	11.8±4.8 ($n=4$)	−3.7±2.9 ($n=3$)	4.1±1.3* ($n=4$)
D-AP5 50 μM ($n=6$)	14.8±2.5	1.8±2.9	1.6±0.7*
CNQX 5 μM ($n=6$)	12.3±1.5	2.6±3.1	9.1±1.9

NMDA, D-AP5 or CNQX were superfused for 8 or 5 min during collection of either basal outflow or electrically-evoked release fractions. Data in column 3 were calculated by subtracting SP-LI basal outflow values in absence of drug from values in presence of drug. Data in column 4 were calculated by subtracting SP-LI basal outflow from evoked release values, both in presence of drug. Statistical analysis between controls and treated slices within the same column was performed by Mann-Whitney *U*-test: *$P<0.01$.

release is both capsaicin-sensitive and voltage-dependent (see Malcangio & Bowery, 1994).

The significant inhibition of the evoked release of SP by NMDA antagonists indicates that there may be a functional NMDA receptor through which glutamate is able to exert a positive control on peptide release. This receptor may be the one identified on presynaptic terminals of primary afferent fibres (Liu *et al*., 1994). In the present study, glutamate was likely to be released, and possibly co-released with SP, from primary afferent fibre terminals (Kangrga & Randic, 1990). Furthermore, it is possible that the amino acid release following dorsal root stimulation was maximal since superfusion with NMDA (1 μM) did not modify electrically-evoked release of SP. However, NMDA did increase basal outflow of SP and a potential source of the peptide would be interneurones which possess NMDA receptors (Greenamyre *et al*., 1984). A higher concentration of NMDA (10 μM) significantly impaired both basal and evoked release of SP, possibly as a consequence of toxic neuronal effects.

In conclusion, this study provides pharmacological evidence for the existence of an NMDA receptor that may be located on SP-containing fibres in the dorsal horn of the spinal cord where it exerts a positive feedback on SP release evoked by C fibre stimulation. These findings add strong interest to the interaction between SP and glutamate and their role in nociceptive mechanisms in the dorsal horn of the spinal cord.

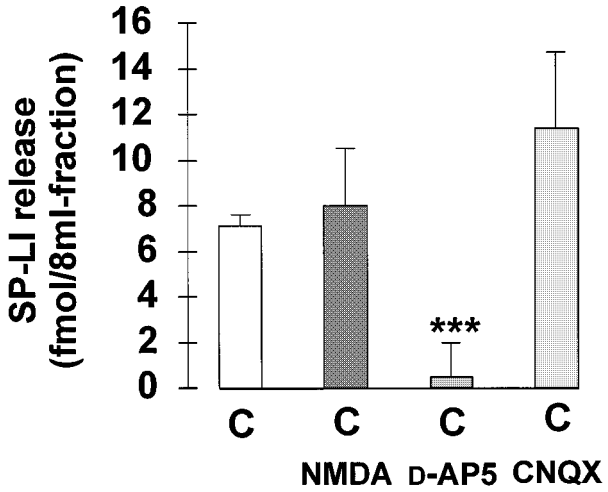

Figure 1 Effect of NMDA, D-AP5 or CNQX on capsaicin-induced release of SP-LI from the isolated spinal cord. D-AP5 and CNQX were present in the 8 min-fraction before capsaicin, during 2 min capsaicin (10 nM) superfusion and in the following 6 min after capsaicin superfusion was terminated. NMDA was superfused for 1 min before capsaicin, during and after capsaicin superfusion. Values are expressed as fmol present in the fraction challenged with capsaicin after subtraction of the basal ouflow: 13.5±1.5 fmol 8 ml-fraction^{-1}. The number of experiments was 4–6. ***$P<0.01$ versus capsaicin (C), Mann-Whitney *U*-test.

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