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Nocistatin reverses nociceptin inhibition of glutamate release from rat brain slices

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Abstract

We have examined the effects of the recently described heptadecapeptide nocistatin on K^+-evoked glutamate release from rat cerebrocortical slices in vitro. In vivo, nocistatin reverses the action of nociceptin. Nocistatin (100 nM, $n = 7$) did not inhibit K^+-evoked glutamate release alone. Nociceptin (100 nM) inhibited glutamate release by $51.7 \pm 8.3\%$ ($P < 0.05$, $n = 6$) and this was fully reversed by nocistatin (100 nM). Nocistatin also appears to be an antagonist of nociceptin action in vitro. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Nociceptin; Nocistatin; Glutamate release

In 1995, Meunier et al. (1995) and Reinsheid et al. (1995) reported the isolation of a heptadecapeptide, nociceptin or orphanin FQ (FGGFTGARKSARKLANQ) respectively, that displayed nanomolar affinity for the orphan opioid-receptor-like receptor, ORL1. Depending on the route of administration the effects of nociceptin on pain threshold are varied, where hyperalgesia, analgesia and antiopioid actions have all been reported (see Henderson and McKnight, 1997; Meunier, 1997). Nociceptin is produced by proteolytic cleavage of a large precursor protein. Two of us (EO-A and SI) have recently described the in vivo effects of a further heptadecapeptide produced from this precursor named nocistatin (TEPGLEEVGEIEQKQLQ). In these studies intrathecal administration of nociceptin produced hyperalgesia and allodynia in mice which was reversed by nocistatin (Okuda-Ashitaka et al., 1998). We have previously shown that nociceptin produces a robust dose-dependent, naloxone insensitive inhibition of glutamate release from rat cerebrocortical slices (Nicol et al., 1996). In the present study we have examined whether nocistatin could reverse nociceptin inhibition of K^+-evoked glutamate release from this preparation.

Wistar rats (200–250 g) were killed by cervical dislocation, the brain rapidly removed and placed in ice-cold oxygenated (95% O_2, 5% CO_2) Krebs buffer pH 7.4, of the following composition (mM): NaCl 115, KCl 4.7, $CaCl_2$ 2.0, $MgCl_2$ 1.2, $NaHCO_3$ 25 and glucose 8.8. The cerebrocortex was cut at right angles to obtain 350×350 μm slices. Approximately 1 ml of washed gravity packed slices were agitated in a shaking water bath at 37°C for 40 min prior to insertion into a perfusion chamber (Nicol et al., 1996) and perfused at a rate of 1 ml/min for 1 h prior to collection of 2 min fractions. A 2 min pulse of 46 mM K^+ (S_1) was applied after 6 min of basal collection, a second pulse (S_2) was applied 30 min after S_1, and fractions collected for a further 8 min after S_2. 100 nM nociceptin and 100 nM nocistatin were added alone or in combination 10 min after the completion of S_1 for the remainder of the experiment. In all experiments 30 μM amastatin, bestatin, captopril and phosphoramidon and 0.1% bovine serum albumin were added 10 min after S_1 to prevent peptide breakdown. Glutamate concentrations in the perfusate were measured fluorimetrically (Nicol et al., 1996). Glutamate release was expressed either, relative to

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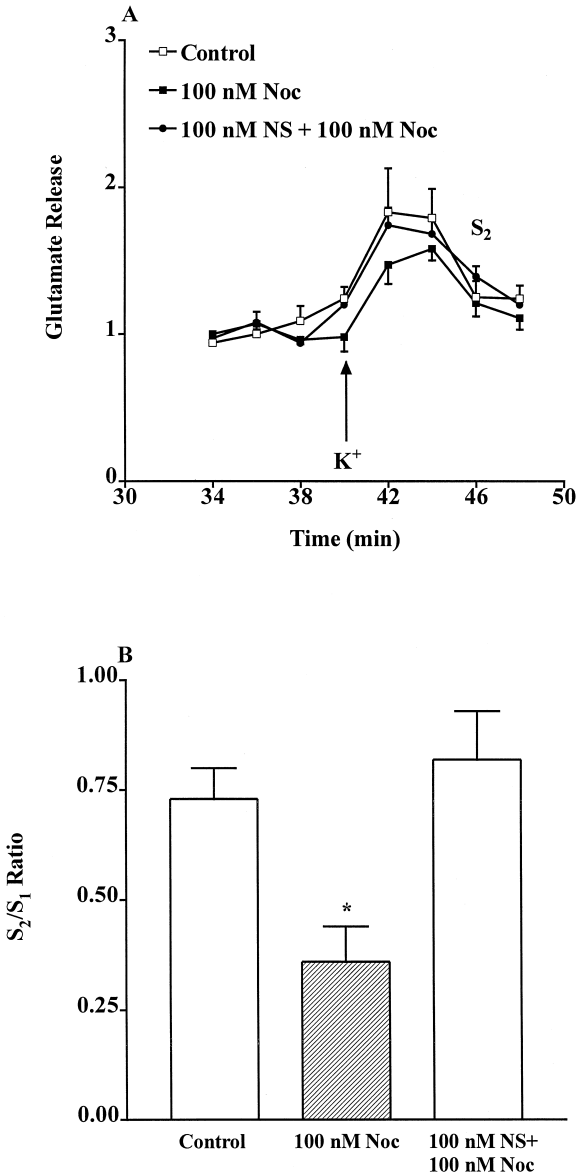

Fig. 1. Nociceptin (Noc) inhibition of glutamate release from rat cerebrocortical slices is reversed by nocistatin (NS). In panel (A) the effect of 100 nM nociceptin alone, and a mixture of 100 nM nociceptin and 100 nM nocistatin applied 10 min after S_1 are shown. The S_1 release profiles have been omitted for clarity but were not significantly different ($P > 0.05$, Friedman analysis). Nociceptin inhibited K^+-evoked glutamate release (control and nociceptin profiles $P < 0.05$, Wilcoxon Rank Sum test) in a nocistatin sensitive manner (control and nociceptin/nocistatin profiles $P > 0.05$, Wilcoxon Rank Sum test). In panel (B) S_2/S_1 ratios from data in (A) are shown. $*$ $P < 0.05$, Wilcoxon Rank Sum test, comparing paired control and nociceptin, and nociceptin and nociceptin/nocistatin combination. All data are mean $\pm$ SEM ($n = 6$) and in (A) glutamate release is expressed relative to the mean of the first three fractions collected during S_2.

the mean of the first three basal samples collected prior to stimulation (S_1 or S_2), or as S_2/S_1 ratio. Data are presented as mean $\pm$ SEM for $n \geq 5$, and statistical comparisons of paired samples were made using Friedman analysis of variance followed by Wilcoxon Rank sum test where appropriate and considered significant when $P < 0.05$.

Depolarisation of rat cerebrocortical slices with 2 min pulses of 46 mM K^+ produced monophasic releases of glutamate for both S_1 and S_2, with a mean S_2/S_1 ratio from 13 control experiments of 0.73 ± 0.05. Nocistatin (100 nM) alone produced no direct inhibition of glutamate release (control S_2/S_1 ratio, 0.73 ± 0.07; nocistatin, 0.62 ± 0.05, $P > 0.05$, $n = 7$). In contrast nociceptin (100 nM) inhibited K^+-evoked glutamate release by $51.7 \pm 8.7\%$ ($P < 0.05$), (Fig. 1A and B), in agreement with our previous data (Nicol et al., 1996). Co-incubation of 100 nM nociceptin with 100 nM nocistatin completely reversed the inhibitory effect of the former peptide (Fig. 1A and B).

We have shown that the novel peptide nocistatin (100 nM) reversed the inhibition produced by nociceptin of K^+-evoked glutamate release from rat cerebrocortical slices, and was itself devoid of any agonist activity. Nociceptin has also been reported to reduce glutamatergic transmission in the neonatal rat hemisected spinal cord preparation (Faber et al., 1996) and to inhibit C-fibre wind-up and post-discharge of spinal dorsal horn neurones of the rat (Stanfa et al., 1996). It will be interesting to determine if nocistatin reverses these effects.

In their initial study Okuda-Ashitaka et al. (1998) reported a reversal of nociceptin (and prostaglandin E_2) induced hyperalgesia and allodynia in vivo. In addition, immunoreactive nocistatin was co-localised with nociceptin immunoreactivity in the rat spinal cord. This is an important observation indicating that nociceptin and nocistatin may be co-released and that both have a role to play in pain transmission. [^{125}I]Lys^{14}(Tyr)nocistatin was found to bind to mouse brain membranes with a K_d of around 5 nM. However, this site was not the ORL1 receptor as nocistatin did not displace [^{3}H]nociceptin binding to recombinant ORL1 receptors expressed in Chinese hamster ovary (CHO) cells. The site at which nocistatin inhibits glutamate release will require further investigation.

Acknowledgements

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