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SPECIAL REPORT

Inhibition by nociceptin of neurogenic inflammation and the release of SP and CGRP from sensory nerve terminals

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Pretreatment with the novel neuropeptide nociceptin (20 μg kg^{-1}, i.p.) caused an inhibition of plasma extravasation evoked by antidromic stimulation of the saphenous nerve or by topical application of 1% mustard oil on the skin of the acutely denervated hindleg of the rat. In contrast, it did not affect non-neurogenic inflammation evoked by s.c. injection of bradykinin after chronic denervation. Release of substance P (SP) and calcitonin gene-related peptide (CGRP) from rat isolated tracheae in response to electrical field stimulation was diminished by nociceptin (100 nM). It is concluded that nociceptin inhibits the release of sensory neuropeptides from terminals of nociceptive neurones.

Keywords: Nociceptin; primary afferent neurones; inflammation; neurogenic inflammation; substance P; calcitonin gene-related peptide; rat trachea

Introduction A novel neurotransmitter biochemically related to opioids has been recently identified. The names suggested for this heptadecapeptide are 'nociceptin' (Meunier *et al*., 1995) and 'orphanin FQ' (Reinscheid *et al*., 1995), because it was found to have nociceptive properties and since its receptor was discovered before the ligand (orphan receptor named ORL_1).

Opioid peptides inhibit neurotransmitter release from the capsaicin sensitive primary afferent nerve terminals (Maggi, 1991). This effect is thought to mediate part of their analgesic action at the level of the spinal cord and underlies the ability of opioids to supress neurogenic inflammation in the periphery. It has been demonstrated recently that nociceptin exerts a prejunctional inhibitory effect on tachykinin-mediated contractions in the guinea-pig renal pelvis due to stimulation of sensory nerves (Giuliani & Maggi, 1996). The aim of the present study was to examine the effect of nociceptin on inflammatory reactions *in vivo* and on substance P (SP) and calcitonin gene-related peptide (CGRP) release *in vitro*.

Methods Rats were anaesthetized with sodium pentobarbitone (40 mg kg^{-1}, i.p.).

In vivo studies Evans blue dye (50 mg kg^{-1}) was injected i.v. to detect and quantify plasma extravasation (Pintér & Szolcsányi, 1995). Nociceptin (20 μg kg^{-1}) or isotonic saline was given i.p. and inflammation was induced 10 min later. Neurogenic inflammation was evoked either by antidromic stimulation of the left saphenous nerve (20 V, 0.5 ms, 5 Hz, 5 min) or by mustard oil (1%) smeared on the right, acutely denervated hindleg. In the latter case bradykinin (0.25 μg, 50 μl) was injected s.c. into the left paw after chronic denervation to induce non-neurogenic inflammation. The animals were exsanguinated 15 min later and the dye content of the inflamed tissue was determined by spectrophotometry. For denervation, saphenous and sciatic nerves were cut 5 days (left leg) and 30 min (right leg) before the experiment.

Release studies Rats were bled and the dissected tracheae of two animals were perfused (1 ml min^{-1}) in an organ bath (1.8 ml) with Krebs solution (95% O_2 and 5% CO_2) at 37°C for 60 min. After the flow was stopped, the solution was changed 3 times for 5 min (prestimulated – stimulated – poststimulated) in the presence and absence of 100 nM nociceptin. Electrical field stimulation (40 V, 0.1 ms, 2 Hz, 50 s) was used (Szolcsányi & Barthó, 1982) to induce peptide release from the tissue pieces. The fractions were collected in ice-cold tubes and the wet weight of each trachea was measured. Specific RIA methods developed in our laboratory were used to determine SP and CGRP concentrations. Detection limits of the radioimmunoassays were 2 fmol/tube (SP) and 1 fmol/tube (CGRP).

Drugs and chemicals Bradykinin, Evans blue dye and CGRP antiserum (Sigma), mustard oil (allylisothiocyanate) (Fluka), sodium pentobarbitone (May and Baker), nociceptin, rat α-CGRP, Tyr-α-CGRP (23–37) (Bachem), SP RIA-tracer (Amersham) and SP antiserum provided by Prof. G.J. Dockray, University of Liverpool were used. ^{125}I-labelled Tyr-α-CGRP (23–37) was prepared in our laboratory. Data are presented as mean ± s.e.mean. Student's *t* tests for paired comparison or independent data were used for statistical evaluation.

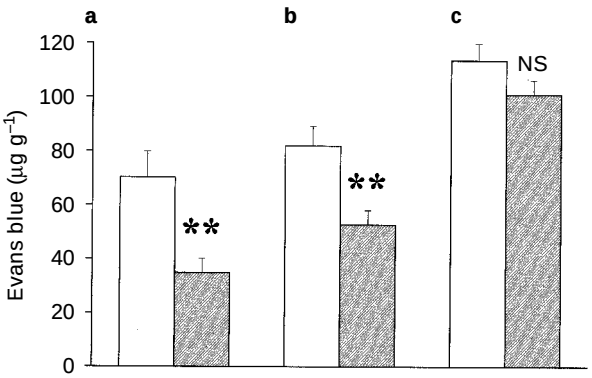

Figure 1 The effect of nociceptin (20 μg kg^{-1}, i.p.) on inflammation in the rat. Neurogenic inflammation in the skin of the hindleg was evoked by (a) antidromic stimulation of the saphenous nerve or (b) by 1% mustard oil after acute denervation. (c) Non-neurogenic inflammation was induced by s.c. injection of bradykinin into the chronically denervated paw. Each value is mean ± s.e.mean of 6–7 experiments. Open columns indicate plasma extravasation in the control (saline-treated) group and hatched columns show Evans blue accumulation after nociceptin pretreatment (**$P<0.01$).

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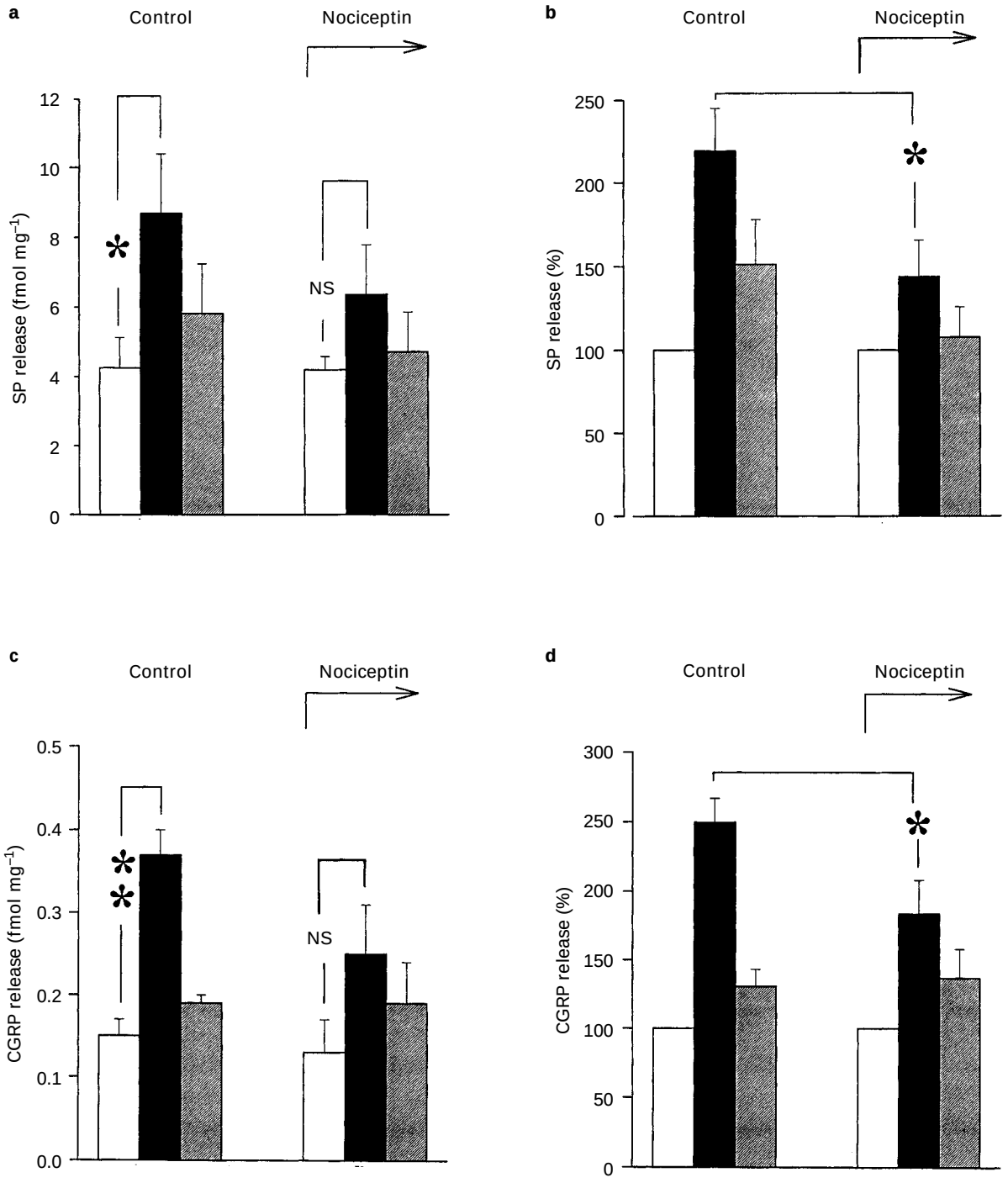

Figure 2 Effect of nociceptin 100 nM on (a and b) substance P (SP) and (c and d) calcitonin gene-related peptide (CGRP) release induced by electrical field stimulation of rat tracheae *in vitro*. Means ± s.e.mean of 7 experiments are shown. Columns refer to the prestimulated (open columns) and poststimulated (hatched columns) period and that during stimulation (solid columns). $^{**}P<0.01$ and $^{*}P<0.05$.

Results *Effect of nociceptin pretreatment on neurogenic and non-neurogenic inflammation* – in vivo *studies* Nociceptin caused 51.39% ($n=6$) inhibition of plasma extravasation induced by antidromic stimulation of the saphenous nerve. When neurogenic inflammation was evoked by chemical stimulation (topical application of 1% mustard oil) this inhibition was 35.7% ($n=7$), while non-neurogenic inflammation evoked by s.c. injected bradykinin ($n=7$) into the chronically denervated paw was not affected by the drug (Figure 1).

Inhibition of SP and CGRP release by nociceptin – in vitro *studies* Electrical field stimulation caused a significant increase of SP and CGRP release from the trachea pieces in the control samples (SP: from 4.26 ± 0.86 to 8.69 ± 1.71 fmol mg^{-1}; CGRP: from 0.15 ± 0.02 to 0.37 ± 0.03 fmol mg^{-1}; $n=7$). In the presence of 100 nM nociceptin the enhancements were inhibited (SP: from 4.20 ± 0.38 to 6.36 ± 1.43 fmol mg^{-1}; CGRP: from 0.13 ± 0.06 to 0.25 ± 0.06 fmol mg^{-1}; $n=7$), but the basal release remained unchanged (Figure 2). If the stimulation-evoked enhancements in peptide release were taken as 100%, inhibition induced by nociceptin for SP and CGRP release corresponded to 63.1% and 44.1%, respectively.

Discussion The present *in vivo* data provide the first evidence for the anti-inflammatory action of nociceptin on neurogenic

inflammation, while non-neurogenic plasma extravasation evoked by bradykinin was not affected. It is concluded that nociceptin inhibits the discharge of sensory neuropeptides from the stimulated sensory nerve endings. In fact, we demonstrated that SP and CGRP released from isolated tracheae in response to electrical stimulation of the capsaicin-sensitive sensory fibres (Szolcsányi & Barthó, 1982) was diminished by nociceptin, while basal release remained unchanged. It has been shown that nociceptin inhibits K^+-evoked glutamate release from rat cerebrocortical slices (Nicol *et al*., 1996) but the cellular mechanism of nociceptin on sensory neurones has not been elucidated. It has been shown that intrathecally given nociceptin inhibits the C-fibre evoked wind-up and post-discharge but not the baseline C-fibre evoked responses of the dorsal horn neurones (Stanfa *et al*., 1996).

In the light of the present biochemical observations the anti-nociceptive role of nociceptin in the spinal cord might be due to its inhibitory effect on stimulation-evoked release of mediators from the central terminals of the nociceptive primary afferent neurones.

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