Relevance of endothelin-1(1-31) synthesis in hind paw nociception mediated by activation of mast cells in mice

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Endothelin-1 (ET-1) is synthesized mainly by cleavage of Big ET-1 by endothelin converting enzyme (ECE). However, Big ET-1 can be also cleaved to ET-1(1-31) by mast cell-derived chymase, which can subsequently be converted to ET-1 by neutral endopeptidase-24.11 (NEP). The present study aimed to assess the ability of ET-1(1-31) to induce nociception and mechanical hypernociception in the hind paw of mice and compare its effects with those evoked by ET-1 and Big ET-1.

Male Swiss mice (n = 6-10) were given intraplantar (i.pl., 20 µl) injections of ET-1 (3 to 30 pmol), ET-1(1-31) (30 to 100 pmol), Big ET-1 (30 to 100 pmol) or vehicle into a hind paw and nociception was evaluated during 30 min, by counting time spent licking the paw. The development of hind paw mechanical hyperalgesia was then evaluated up to 4 h after injection, using von Frey filaments and the Up and Down Dixon method. Other mice were given i.pl. injections of thiorphan (NEP inhibitor, 300 nmol), phosphoramidon (NEP and ECE inhibitor, 100 nmol), chymostatin (chymase inhibitor,100 nmol) or vehicle 15 min prior to a second i.pl. injection of ET-1 (10 pmol), ET-1(1-31) (10 pmol), Big ET-1 (30 pmol) or vehicle and evaluated for nociceptive and hyperalgesic responses. Additional groups of mice were pretreated with i.pl. injections of thiorphan (300 and 1000 nmol), phosphoramidon, chymostatin or vehicle, 15 min prior to co-injection of Big ET-1 (30 pmol) plus non-nociceptive doses of compound 48/80 (0.1 µg) or ovalbumin (OVA, 0.05 µg; in mice sensitised to OVA). In some animals, the enzymatic inhibitors were given together with BQ-123 (ETA receptor antagonist, 10 nmol) or BQ-788 (ETB receptor antagonist, 10 nmol) 15 min prior to challenge with nociceptive doses of 48/80 (1 µg) or OVA (0.3 µg). The study was approved by UFSC's Ethics Committee on Animal Use.

ET-1, ET-1(1-31) or Big ET-1 induced graded nociception and mechanical hyperalgesia. Thiorphan inhibited nociception induced by ET-1(1-31) only. Phosphoramidon reduced nociception induced by ET-1(1-31) and Big-ET-1, but not ET-1. Chymostatin failed to alter the nociception induced by ET-1(1-31), Big ET-1 or ET-1. The association of Big ET-1 together with sub-threshold nociceptive doses of compound 48/80 or OVA increased the nociception evoked by Big ET-1 by 93% and 154%, respectively. Nociception induced by the combinations of Big ET-1 plus compound 48/80 or OVA was reduced by phosphoramidon or chymostatin pretreatment, while thiorphan was only effective at 1000 nmol. Nociceptive responses to an effective dose of compound 48/80 were reduced by BQ-123, chymostatin, thiorphan, but not BQ-788 or phosphoramidon, while those to OVA were reduced by all pretreatments (thiorphan not tested).

These results suggest that ET-1 formed via an alternative synthesis pathway, which involves formation of the intermediary peptide ET-1(1-31), contributes significantly to nociception induced by the activation of mast cells.

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