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Mechanisms underlying the nociceptive and inflammatory responses induced by trypsin in the mouse paw

Ana Flávia Paszcuk [a], Nara L.M. Quintão [a], Elizabeth S. Fernandes [a], Luiz Juliano [b], Kevin Chapman [d], Patricia Andrade-Gordon [e], Maria Martha Campos [c], Nathalie Vergnolle [d], João B. Calixto [a,*]

[a] *Department of Pharmacology, Centre of Biological Sciences, Universidade Federal de Santa Catarina UFSC, Campus Universitário, Trindade, 88049-900, Florianópolis, SC, Brazil*
[b] *Department of Biophysics, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, Brazil*
[c] *School of Dentistry, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Brazil*
[d] *Department of Pharmacology and Therapeutics, University of Calgary, Calgary, Alberta, Canada*
[e] *RW Johnson Pharmaceutical Research Institute, Spring House, Pennsylvania, USA*

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Abstract

It has been demonstrated that trypsin is able to evoke the classical signals of inflammation, mainly via the activation of proteinase-activated receptor-2 (PAR-2). This study was designed to evaluate the inflammatory and nociceptive responses caused by trypsin injection in the mouse paw. Trypsin produced a dose- and time-related paw edema, a response that was markedly reduced in PAR-2-deficient mice compared to wild-type mice, particularly at the early time-points after trypsin injection. In addition, trypsin produced an increase in myeloperoxidase (MPO) activity, which was significantly reduced in PAR-2-deficient mice. The injection of trypsin into the mouse paw also elicited a dose- and time-dependent spontaneous nociception, as well as thermal and mechanical hypernociceptive responses, which were consistently decreased in mice with genetic deletion of PAR-2. Pharmacological evaluation revealed that edema formation and spontaneous nociception caused by trypsin injection in the mouse paw are mediated by a complex range of mediators. Both edema and nociception seem to rely on the production of neuropeptides, probably involving C-fibre activation and vanilloid receptor-1 (TRPV1), besides the stimulation of kinin B_2 receptors. Edematogenic response is also likely related to the production of cyclooxygenase (COX) metabolites, whereas the mast cell activation appears to be greatly associated to spontaneous nociception. Altogether, the present results indicate that trypsin-induced edema and nociception in the mouse paw represent multi-mediated responses that are largely, but not exclusively, related to the activation of PAR-2. These pieces of evidence provide new insights on the role of trypsin in pain and inflammation.

Keywords: Trypsin; Inflammation; Edema; Nociception; PAR-2; (Mouse)

1. Introduction

Proteases display several biological actions, including the degradation of proteins and the control of the cell cycle. Accumulating evidence indicates that serine proteases exert their cellular functions by activating specific G protein-coupled receptors, named 'proteinase-activated receptors' (PARs) (MacFarlane et al., 2001; Schmidlin and Bunnett, 2001; Ossovskaya and Bunnett, 2004). PARs are activated via a process that involves recognition of the receptor by the enzyme, the cleavage at a specific enzymatic site within the extracellular NH_2-terminus of the receptor, and finally the exposure of a new NH_2-terminus sequence, that in turn acts as an anchored receptor-stimulating ligand (usually comprised by six or more amino acids) to activate the cleaved receptor molecule (MacFarlane et al., 2001; Schmidlin and Bunnett, 2001). To date, four members of this family have been cloned (PAR-1 to PAR-4) from rodents and humans (MacFarlane et al., 2001; Ossovskaya and Bunnett,

* Corresponding author. Tel.: +55 48 3721 9491; fax: +55 48 3337 5479.
E-mail addresses: calixto@farmaco.ufsc.br, calixto3@terra.com.br (J.B. Calixto).

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2004). Thrombin is a known activator of PAR-1, PAR-3 and PAR-4, whereas the coagulation factors VIIa and Xa activate PAR-1 and PAR-2. Mast cell tryptase activates PAR-2, and neutrophil cathepsin G stimulates PAR-4. Finally, trypsin (from both pancreatic and extra-pancreatic sources) is able to preferentially activate PAR-2 and to a lesser extent PAR-1 and PAR-4 (MacFarlane et al., 2001; Schmidlin and Bunnett, 2001). Apart from PAR-3, the PARs can be activated by short synthetic peptides of 5 or 6 amino acids that mimic the anchored receptor-stimulating ligands (Schmidlin and Bunnett, 2001).

Trypsin is one of the best-characterized serine proteinases, which is produced as a zymogen (trypsinogen) in the acinar cells of the pancreas. It is transported to the duodenum and is transformed into the mature form by enterokinases, acting as an essential food-digestive enzyme (Dunn, 1989). Trypsin is widely expressed in several tissues, such as vascular endothelial cells of humans or epithelial cells of the skin, esophagus, stomach, small intestine, large intestine, lung, kidney, liver and extrahepatic bile duct, as well as in leukocytes of the spleen and neurons, from both humans and mice (Koshikawa et al., 1998). Regarding the inflammatory scenario, it has been recently demonstrated that trypsin, probably via PAR-2 activation, induces vasodilatation and extravasation of plasma proteins in rats (Obreja et al., 2006). Trypsin is also able to stimulate tumor necrosis factor α (TNFα) secretion from rat peritoneal macrophages (Lundberg et al., 2000), to cause nitric oxide (NO)-dependent vasodilation in rats (Kawabata et al., 2001), and to potently induce joint inflammation in mice (Kelso et al., 2006). Moreover, at sub-inflammatory doses, the synthetic PAR-2 agonist provokes thermal and mechanical hyperalgesia, as well as spinal nociceptor activation (Vergnolle et al., 2001).

The present study was designed to evaluate the mechanisms and mediators involved in trypsin-evoked edema and nociceptive alterations (spontaneous, thermal and mechanical hypernociception), when injected locally into the mouse paw. Attempts have also been made in order to determine the ability of trypsin to induce neutrophil migration, by measuring the myeloperoxidase (MPO) activity in the mouse paw.

2. Materials and methods

2.1. Animals

Most experiments were performed using male Swiss mice weighing 20–30 g, kept in controlled temperature (22±2 °C) and humidity (60–70%), under a 12 h light–dark cycle. Food and water were freely available. Animals were acclimatized to the laboratory for at least 1 h before testing and were used once throughout the experiments. The studies reported in this manuscript followed the "Principles of Laboratory Animal Care" from NIH publication No. 85-23 and ethical guidelines for investigation of experimental pain in conscious animals (Zimmermann, 1983). The Ethics Committee of the Universidade Federal de Santa Catarina approved all the experimental procedures described herein (protocol numbers 262/CEUA and 23080.035334/2003-16/UFSC). The number of animals and intensity of noxious stimuli used were the minimum necessary to demonstrate the consistent effects of the drug treatment. PAR-2-deficient mice (PAR-2$^{-/-}$) and wild-type littermates (C57Bl6 background, used as the control group) were bred at the University of Calgary animal care facility and were originally from RW Johnson.

2.2. Paw edema evoked by trypsin

The experiments were conducted according to the procedures described before (da Cunha et al., 2004). Edema was induced in the right hindpaw by a 20-μl intraplantar (i.pl.) injection of trypsin (3–300 μg/paw, prepared in saline). The contralateral paw (left paw) received 20 μl of saline and it was used as the control. Edema was measured with a plethysmometer (Ugo Basile) or with an electronic calliper (measuring paw diameter for PAR-2$^{-/-}$ and littermates), at several time-points (10–50 min and 1–6 h) after injection of trypsin. Edema was expressed as the difference between the right and left paws. All experimental procedures were performed under light inhalation anaesthesia with oxygen (2%) and isoflurane (3%).

2.3. Trypsin-induced nociception

After an adaptation period, each mouse received a 20-μl i.pl. injection of trypsin (30–600 μg/paw) into the right hindpaw. Control animals received saline (20 μl) by i.pl. route. The mice were observed individually for 10 min following trypsin injection. The amount of time (in seconds) spent licking and/or biting the injected paw was recorded with a chronometer and it was considered as indicative of spontaneous nociception.

Nociceptive responses to thermal stimulus were evaluated by measuring paw withdrawal latency in response to a radiant heat stimulus using a plantar test apparatus (Ugo Basile, Milan, Italy). Nociceptive responses to mechanical stimulation were performed using von Frey filaments with bending forces of 0.4 g (3.61) and 0.6 g (3.84). Mice were placed individually in clear plastic boxes with a metal grid floor and were allowed to acclimatize for several minutes. The filaments were applied three times randomly for 1 s. A score is assigned based on the mouse's response: 0 = no movement, 1 = removal of the paw, 2 = removal of the paw and vocalisation, licking or holding of the paw. Mechanical nociceptive scores are expressed as a percentage of the maximal score for the three applications. Measurements were performed before (time 0) and at different times after trypsin intraplantar injection.

2.4. Granulocyte recruitment: MPO assay

Granulocyte recruitment in the mouse paw was assessed indirectly by means of tissue MPO activity according to the method described beforehand (Fernandes et al., 2005). For this purpose, animals received a 20-μl i.pl. injection of trypsin (30 μg/paw) into the right paw. Saline-injected paws were used as control. Animals were sacrificed at different time-points (1–12 h) and the subcutaneous tissue of the paws was removed, homogenized at 5% (w/v) in EDTA/NaCl buffer (pH 4.7) and centrifuged at 4400 ×*g* for 15 min at 4 °C. The pellet was re-