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Short communication

Suppression by protease-activated receptor-2 activation of gastric acid secretion in rats

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Abstract

Activation of protease-activated receptor-2 (PAR-2), a receptor activated by trypsin/tryptase, induces neurally mediated gastric mucus secretion accompanied by mucosal cytoprotection. In the present study, we investigated whether PAR-2 could modulate gastric acid secretion in rats. Messenger RNAs for PAR-2 and PAR-1 were detected in the gastric mucosa and smooth muscle. The PAR-2-activating peptide SLIGRL-NH_2, but not the inactive control peptide, when administered i.v., strongly suppressed gastric acid secretion in response to carbachol, pentagastrin or 2-deoxy-D-glucose in the rats with a pylorus ligation. The PAR-2-mediated suppression of acid secretion was resistant to cyclooxygenase inhibition or ablation of sensory neurons by capsaicin. Our results provide novel evidence that in addition to stimulating neurally mediated mucus secretion, activation of PAR-2 suppresses gastric acid secretion independently of prostanoid production or sensory neurons. These dual actions of PAR-2 would result in gastric mucosal cytoprotection.

Keywords: PAR-2 (protease-activated receptor-2); Protease (proteinase); Gastric acid secretion; Gastric mucosal cytoprotection

1. Introduction

Protease-activated receptor-2 (PAR-2) (Nystedt et al., 1994), a G protein-coupled receptor, is activated by proteolytic unmasking of the N-terminal extracellular tethered ligand that presumably binds to the extracellular loop 2 of the receptor itself (Lerner et al., 1996). Trypsin, mast cell tryptase and coagulation factors VIIa and Xa have been identified as endogenous activators for PAR-2 (Molino et al., 1997; Camerer et al., 2000; Kawabata et al., 2001d). PAR-2 can also be non-enzymatically activated by synthetic peptides based on the receptor-activating sequence of the tethered ligand (e.g., SLIGRL for murine PAR-2) (Nystedt et al., 1994). PAR-2 is involved in a variety of biological events. PAR-2 plays a dual role, in modulation of smooth muscle motility in vitro and in vivo, being excitatory and also inhibitory (Saifeddine et al., 1996; Corvera et al., 1997; Cocks et al., 1999a,b; Kawabata et al., 1999, 2001c). PAR-2 also mediates salivary and pancreatic secretion (Nguyen et al., 1999; Kawabata et al., 2000). PAR-2 is also expressed in sensory neurons and involved in neurogenic inflammation (Steinhoff et al., 2000) and nociception (Kawabata et al., 2001a; Vergnolle et al., 2001). In contrast, the PAR-2 agonist, given in vivo, induces cytoprotective mucus secretion in the gastric mucosa, by stimulating sensory neurons (Kawabata et al., 2001b). PAR-2 thus plays a dual role, which might be protective following its mild activation but pro-inflammatory/nociceptive when activated excessively (Kawabata et al., 2001a,b). In the present study, we focused on the protective role of PAR-2 in the stomach, and examined if activation of PAR-2 could inhibit acid secretion in the stomach. Here we describe, for the first time to our knowledge, that PAR-2 functions to suppress gastric acid secretion in a manner independent of prostanoids and capsaicin-sensitive sensory neurons.

2. Materials and methods

2.1. Animals care and use

Male Wistar rats (7 weeks old, Japan SLC, Japan) were used according to the Japanese Pharmacological Society's

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Animal Guiding Principles for the Care and Use of Laboratory Animals, and with approval from the institutional committee for ethics.

2.2. Detection of PAR-1 and PAR-2 mRNAs by reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNAs were isolated from freshly dissected rat gastric mucosa and smooth muscle tissues using the TRI reagent (Molecular Research Center, Cincinnati OH, USA). The RNA was reverse-transcribed with a first strand cDNA synthesis kit using pd(N)6 primer (Pharmacia LKB Biotechnology, Uppsala, Sweden) according to manufacturer's recommendations at 37 °C for 60 min. In PCR amplification, the primers used for PAR-1 were: 5′-AAAAGCTTCCCGCTCATTTTTTCTCAGGAA-3′ and 5′-GGGAATTCAATCGGTGCCGGAGAAGT-3′. The primers for PAR-2 were: 5′-CAACAGTAAAGGGAGAAGTC-3′ and 5′-AGCACATC(A/G)TGACAGGT(A/G)GTG-3′. The PCR signals yielded by the PAR primers were normalized to the PCR signal generated by the primers for actin, 5′-CGTGGGCCGCCCTAGGCACCA-3′ and 5′-TTGGCCTTAGGGTTCAGGGGG-3′. Routinely, amplification was obtained using 2.5 units of *Taq* DNA polymerase (Promega, Madison, WI) in a 10 mM Tris–HCl buffer, pH 9.0 (50 ml, final volume) containing $MgCl_2$ (1.5 mM), KCl (50 mM), 0.1% v/v Triton X-100, and 0.2 mM each of deoxynucleotide triphosphates. The amplification reaction was allowed to proceed for 35 cycles, beginning with a 1-min denaturing period at 94 °C, followed by a 1-min reannealing time at 55 °C, then a 1-min primer extension period at 72 °C. The PCR products were separated by 1.5% agarose gel electrophoresis and visualized by ethidium bromide.

2.3. In vivo assay of gastric acid secretion

Rats were deprived of food, but not water, for 24 h before the experiments. Under ether anaesthesia, the pylorus of the laparotomized rat was exposed and ligated with silk, care being taken to avoid local nerve and vascular trauma. Immediately after quick suturation, the rat received s.c. carbachol at 60 μg/kg, i.p. pentagastrin at 120 μg/kg or i.v. 2-deoxy-D-glucose at 200 mg/kg. Amastatin, an inhibitor of aminopeptidease, a degradative enzyme for peptides, at 2.5 μmol/kg, was administered i.v. 1 min after injection with secretagogues, and the specific PAR-2 agonist SLIGRL-NH_2 at 0.25–5 μmol/kg, the inactive control peptide LSIGRL-NH_2 at 5 μmol/kg or vehicle (saline) was administered i.v. after an additional 1 min. The rats were sacrificed by decapitation 30 min after administration of peptides. The luminal liquid was collected from the excised stomach and filtered though gauze to remove residual debris. Acid content in the collected samples was determined by titration to pH 7.0 with 5% sodium bicarbonate. Results are expressed as the amount (μmol) of acid accumulated for 30 min.

2.4. Inhibition experiments

The rats received i.p. indomethacin, an inhibitor of cyclooxygenase, at 30 mg/kg, 30 min before i.v. SLIGRL-NH_2 at 5 μmol/kg. In the experiments to test involvement of sensory neurons, the rats received three doses of capsaicin (25, 50 and 50 mg/kg, s.c.) over 32 h (at 0, 6, 32 h, respectively) under pentobarbital (30 mg/kg, i.p.) anaesthesia (Steinhoff et al., 2000), 10 days before experiments.

2.5. Peptides and other chemicals

PAR-2-related peptides were prepared by standard solid phase synthesis procedures. The concentration, purity and composition of the peptides were determined by high-performance liquid chromatography, mass spectrometry and quantitative amino acid analysis. Carbachol, pentagastrin, 2-deoxy-D-glucose and capsaicin were purchased from Sigma (St. Louis, MO, USA), and indomethacin was from Wako (Osaka, Japan). Amastatin was obtained from Peptide Institute (Minoh, Japan). Indomethacin was dissolved in 4% sodium bicarbonate, and capsaicin was in a saline solution containing 10% ethanol and Tween 80. Other drugs including peptides were dissolved in saline. Control animals received administration of vehicle.

2.6. Statistics

Data are expressed as mean±S.E.M. Statistical analysis was performed using the Tukey's multiple comparison test or Student's *t*-test, and set at a $P<0.05$ level.

3. Results

The PCR signals for mRNAs for PAR-2 and PAR-1 were detected abundantly in both the gastric mucosa and smooth muscle. The RT-PCR analysis, though not highly quantitative, showed that the intensity of the signal for PAR-2 was stronger than that for PAR-1 in the mucosa, while an opposite profile was obtained in the smooth muscle (Fig. 1A).

SLIGRL-NH_2 at 0.25, 1 and 5 μmol/kg, when administered i.v. in combination with amastatin at 2.5 μmol/kg, produced a dose-dependent decrease in the gastric acid secretion in response to carbachol in the rats. In contrast, LSIGRL-NH_2, a PAR-2-inactive peptide, at 5 μmol/kg, in combination with amastatin, had no such effect (Fig. 1B). In preliminary experiments, SLIGRL-NH_2 was less effective without preadministration of amastatin (data not shown). It was also confirmed that amastatin alone at 2.5 μmol/kg did not alter the acid secretion; the carbachol-evoked acid output (μmol/30 min) in the rats treated with vehicle and amastatin was 166.6±20.9 and 171.6±24.1 (n=11), respectively. Furthermore, SLIGRL-NH_2, administered at 5 μmol/kg in combination with amastatin, strongly suppressed the

acid secretion produced by pentagastrin (Fig. 1C) and by 2-deoxy-D-glucose (Fig. 1D).

The inhibition by the PAR-2 agonist SLIGRL-NH_2 at 5 μmol/kg in combination with amastatin of carbachol-induced gastric acid secretion was not reduced by indomethacin at 30 mg/kg (Fig. 2A). Ablation of sensory neurons by pretreatment with capsaicin, by itself, tended to decrease the gastric acid secretion, an effect being not statistically significant. The capsaicin treatment failed to alter the anti-secretory effect of the PAR-2 agonist at the same dose in combination with amastatin (Fig. 2B).

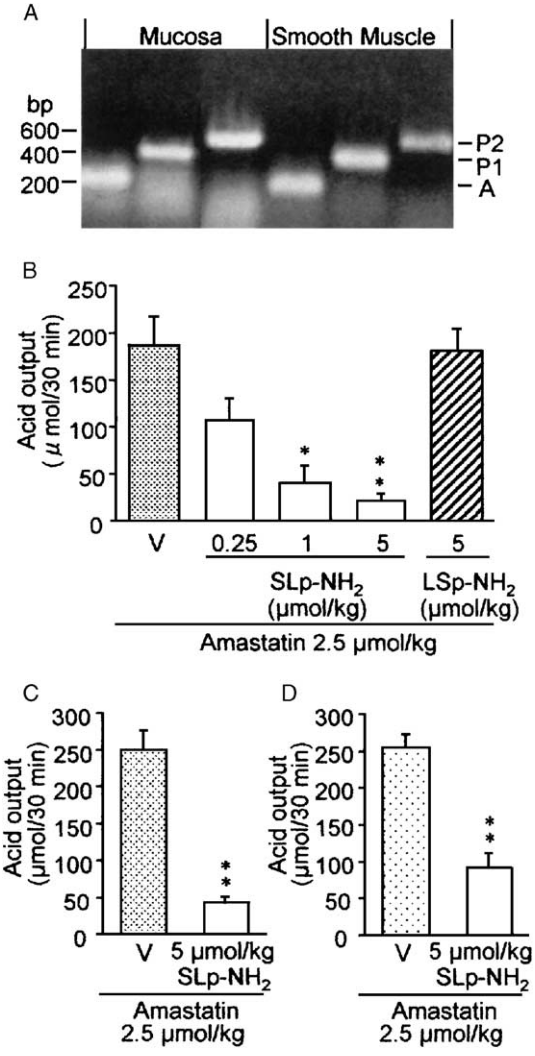

Fig. 1. Suppression of gastric acid secretion by PAR-2 in the rat and RT-PCR detection of PAR-2 mRNAs in the rat stomach. (A) RT-PCR analysis of PAR-1 and PAR-2 mRNAs in the rat gastric mucosa and smooth muscle. Right, positions of the PCR products for PAR-1 (P1), PAR-2 (P2), and actin (A). Left, positions of the oligonucleotide markers (in bp). (B, C and D) Effect of the PAR-2 agonist on gastric acid secretion induced by carbachol (B), pentagastrin (C) or 2-deoxy-D-glucose (D) in the rat. The PAR-2 agonist SLIGRL-NH_2 (SLp-NH_2) or the control peptide LSIGRL-NH_2 (LSp-NH_2), in combination with amastatin at 2.5 μmol/kg, were administered i.v. to rats. Data represent the mean±S.E.M. from 20 (vehicle) or 6–9 (peptides) rats (B) and from 8 (vehicle) or 4 (peptide) rats (C, D). *P<0.05, **P<0.01 vs. vehicle (V).

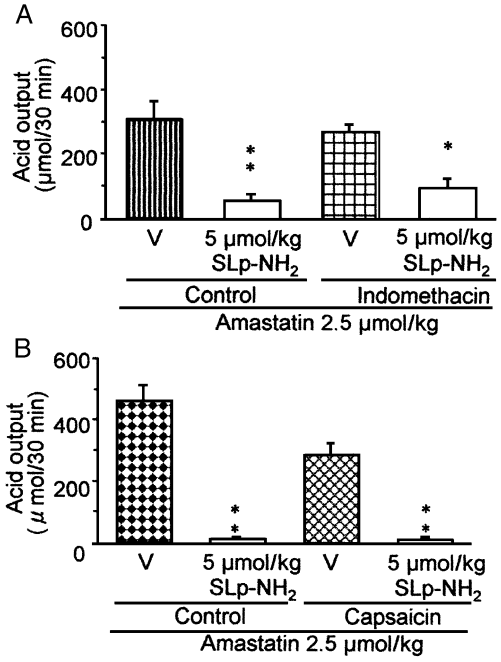

Fig. 2. Lack of effects of indomethacin (A) and capsaicin (B) on inhibition by the PAR-2 agonist of carbachol-induced gastric acid secretion. Indomethacin was administered i.p. 30 min before i.v. challenge with the PAR-2 agonist SLIGRL-NH_2 (SLp-NH_2), in combination with amastatin at 2.5 μmol/kg (A). SLIGRL-NH_2 in combination with amastatin was also administered in the same manner to the rats that had received repeated doses of capsaicin (B), as described in Materials and methods. Data represent the mean±S.E.M. from eight to nine rats. *P<0.05, **P<0.01 vs. the respective vehicle-treated group (V).

4. Discussion

To our knowledge, the present study demonstrates, for the first time, that activation of PAR-2 inhibits gastric acid secretion induced by carbachol, pentagastrin or 2-deoxy-D-glucose, an effect being independent of prostanoid formation and capsaicin-sensitive sensory neurons. This effect, in addition to the neurally mediated mucus secretion (Kawabata et al., 2001b), would contribute to the gastric mucosal cytoprotection exerted by PAR-2 (Kawabata et al., 2001b).

PAR-2 appears to play a general and/or key role in regulation of digestive exocrine secretion. PAR-2 triggers salivation and secretion of pancreatic juice including amylase (Nguyen et al., 1999; Kawabata et al., 2000), independently of sensory neurons (Kawabata et al., 2001b, manuscript in preparation). In contrast, the PAR-2 agonist induces gastric mucus secretion by activating sensory neurons in the stomach (Kawabata et al., 2001b). Calcitonin gene-related peptide (CGRP) that can be released from the sensory neurons following PAR-2 activation (Steinhoff et al., 2000; Kawabata et al., 2001b) is capable of inhibiting secretion of gastric acid (Abdel-Salam et al., 1999b), leading us to the hypothesis that

the PAR-2 agonist might inhibit acid secretion by a neuronal mechanism. Although our data, in keeping with this hypothesis, did indeed demonstrate a PAR-2-mediated suppression of acid secretion, this effect was unexpectedly independent of capsaicin-sensitive sensory neurons. The fact that capsaicin treatment, by itself, produced slight inhibition of the acid secretion (Fig. 2B), has yet to be interpreted, although the effects of capsaicin alone on gastric acid secretion seem very complex, varying with experimental models employed (Abdel-Salam et al., 1999a). The lack of effect of indomethacin in our study also excludes the possibility of involvement of prostanoids, known to inhibit acid secretion (Ballinger, 1994). Such prostanoids can be generated following PAR-2 activation in certain tissues or cells (Saifeddine et al., 1996; Cocks et al., 1999a). The experiments to elucidate the precise mechanisms for the PAR-2-mediated suppression of acid secretion are now in progress in our laboratory. It will be necessary to determine in which cell types PAR-2 are expressed, in relation to the inhibitory effect on the acid secretion from parietal cells.

Our previous study has shown that the PAR-2 agonist strongly reduces the gastric mucosal lesion produced by either HCl–ethanol or indomethacin (Kawabata et al., 2001b). Interestingly, the PAR-2 agonist produced a strong cytoprotective effect at relatively low doses, whereas this effect decreased at high doses (Kawabata et al., 2001b). These findings might reflect a pro-inflammatory action of PAR-2 agonists when locally administered at high doses as described elsewhere (Kawabata et al., 1998; Steinhoff et al., 2000). Taken together with the present findings, we believe that PAR-2 agonists, as long as used at appropriately low doses, could be therapeutically useful for the treatment of gastric ulcer disease.

The present study provides novel evidence for a cytoprotective role for PAR-2 through suppression of gastric mucosal acid secretion, suggesting further that PAR-2 could be a novel target for development of anti-ulcer drugs.

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