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Effects of trypsin, thrombin and proteinase-activated receptors on guinea pig common bile duct motility

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ABSTRACT

Trypsin and thrombin activate proteinase-activated receptors (PARs), which modulate gastrointestinal motility. The common bile duct is exposed to many proteinases that can activate PARs, especially during infection and stone obstruction. We investigated PAR effects on common bile duct motility in vitro. Contraction and relaxation of isolated guinea pig common bile duct strips caused by PAR_1, PAR_2 and PAR_4 agonists were measured using isometric transducers. Reverse transcription polymerase chain reaction (RT-PCR) was performed to determine the expression of PAR_1 and PAR_2. Thrombin and two PAR_1 peptide agonists, TFLLR-NH_2 and SFLLRN-NH_2, evoked moderate relaxation of the carbachol-contracted common bile duct in a concentration-dependent manner. Trypsin and three PAR_2 peptide agonists, 2-furoyl-LIGRLO-NH_2, SLIGKV-NH_2 and SLIGRL-NH_2, generated moderate to marked relaxation as well. The existence of PAR_1 and PAR_2 mRNA in the common bile duct was identified by RT-PCR. Moreover, two PAR_4-selective agonists, AYPGKF-NH_2 and GYPGQV-NH_2, produced relaxation of the common bile duct. In contrast, all PAR_1, PAR_2 and PAR_4 inactive control peptides did not elicit relaxation. This indicates that PAR_1, PAR_2 and PAR_4 mediate common bile duct relaxation. The thrombin, TFLLR-NH_2, trypsin, and AYPGKF-NH_2-induced responses were not affected by tetrodotoxin, implying that the PAR effects are not neurally mediated. Our findings provide the first evidence that PAR_1 and PAR_2 mediate whereas agonists of PAR_4 elicit relaxation of the guinea pig common bile duct. Trypsin and thrombin relax the common bile duct. PARs may play an important role in the control of common bile duct motility.

1. Introduction

Trypsin and thrombin activate proteinase-activated receptors (PARs) through proteolysis. The proteolysis of N-terminals of PARs by proteinases unmasks new terminal sequences which activate PARs [1–5]. Four subtypes of PARs, i.e. PAR_1, PAR_2, PAR_3 and PAR_4, have been described. The tethered peptide ligands are SFLLR, SLIGKV, and GYPGQV for human, as well as SFFLR, SLIGRL, and GYPGKF for mouse PAR_1, PAR_2, and PAR_4, respectively. On the other hand, the ability of the PAR_3 tethered peptide, TFRGAP, to stimulate PAR_3 is unclear [1]. Thrombin is a main activator of PAR_1, PAR_3 and PAR_4 and trypsin, of PAR_2 [1–4].

PARs are found in various tissues, including the gastrointestinal tract [1–4,6]. PAR_1, PAR_2 and PAR_4 modulate the gastrointestinal motility and secretion [1,6–14]. In the human and guinea pig gallbladder, both PAR_1 and PAR_2 mediate contraction; however, PAR_4 activation does not alter gallbladder motility [10,11]. The PAR effects on the common bile duct (CBD) motility are not known. We hypothesized that PAR activation might cause contraction or relaxation of the CBD, similar to the gallbladder. The CBD is exposed to many proteinases that can activate PARs, especially during CBD infection and stone obstruction. Pancreatic trypsin, extravasated plasma thrombin and bacterial proteinases may activate PARs [3,6,10]. Because CBD motility could affect bile flow, stasis of which may promote the formation of CBD stone [15,16], it is important to study the effects of PARs on CBD motility. The purpose of the present study was to investigate the effects mediated by PAR_1, PAR_2 and PAR_4 on CBD motility. We found that activation of PAR advocates relaxation but not contraction of the CBD, which is the opposite of the PAR effects in the gallbladder.

2. Materials and methods

Male Hartley guinea pigs (300–350 g) were obtained from the National Laboratory Animal Center, Taiwan. All procedures were performed in compliance with relevant laws and institutional guidelines and approved by the Institutional Animal Care and Use Committee of E-Da Hospital. Thrombin (from bovine plasma), trypsin (from porcine pancreas, type IX-S), amastatin, carbachol, and all buffer reagents were purchased from Sigma Chemical, St Louis, MO, USA; TFLLR-NH_2 (Thr-Phe-Leu-Leu-Arg-NH_2, selective PAR_1 agonist), SFLLRN-NH_2 (Ser-Phe-Leu-Leu-Arg-Asn-NH_2, PAR_1 agonist), RLLFT-NH_2 (Arg-Leu-Leu-Phe-Thr-NH_2, inactive PAR_1 control peptide), SLIGRL-NH_2 (Ser-Leu-Ile-Gly-Arg-Leu-NH_2, selective PAR_2 agonist), 2-furoyl-LIGRLO-NH_2 (2-furoyl-Leu-Ile-Gly-Arg-Leu-Orn-NH_2, selective PAR_2 agonist),

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GYPGQV-NH_2 (Gly-Tyr-Pro-Gly-Gln-Val-NH_2, selective PAR_4 agonist), and AYPGKF-NH_2 (Ala-Tyr-Pro-Gly-Lys-Phe-NH_2, selective PAR_4 agonist) were obtained from American Peptide Company, Sunnyvale, CA, USA. SLIGKV-NH_2 (Ser-Leu-Ile-Gly-Lys-Val-NH_2, selective PAR_2 agonist) and VKGILS-NH_2 (Val-Lys-Gly-Ile-Leu-Ser-NH_2, inactive PAR_2 control peptide) were purchased from Bachem, Bubendorf, Switzerland. YAPGKF-NH_2 (Tyr-Ala-Pro-Gly-Lys-Phe-NH_2, inactive PAR_4 control peptide) was purchased from Peptides International, Louisville, Kentucky, USA. Tetrodotoxin was obtained from Tocris, Cookson, Avonmouth Bristol, UK. Reverse transcription polymerase chain reaction (RT-PCR) reagents and primers for guinea pig PAR_1 and PAR_2 were purchased from Invitrogen, Carlsbad, CA, and Integrated DNA Technologies, Inc., Coralville, IA, USA, respectively.

2.1. Measurement of contraction and relaxation of guinea pig CBD muscle strips

Measurements of contraction and relaxation of isolated muscle strips from the guinea pig CBD were performed according to the procedure published previously [17–19] with minor modifications [20]. In brief, the guinea pig was euthanized by CO_2 and the CBD, from the outer surface of the duodenum to the junction with the hepatic duct, was removed and placed in standard incubation solution, containing 118 mM NaCl, 25 mM $NaHCO_3$, 4.7 mM KCl, 14 mM glucose, 1.2 mM NaH_2PO_4, 1.8 mM $CaCl_2$, gassed with 95% O_2–5% CO_2. The final pH at 37 °C was 7.40 ± 0.05. The CBD was cut longitudinally to make a 0.3 × 1.0 cm strip, which was attached along the longitudinal axis to an organ bath using surgical silk sutures and incubated at 37 °C in the standard incubation solution continuously gassed with 95% O_2–5% CO_2. The strip was connected to an isometric transducer (FT.03; Grass Technologies, West Warwick, RI, USA), which was connected to an integrated amplifier and computer recording system (BIOPAC systems, Goleta, CA, USA). The basal tension of the muscle strip was adjusted to 0.5 g. Experiments were started after a 45-min equilibration period. The PAR related agents were added to resting or carbachol-contracted muscle strips. All PAR agonists did not stimulate contraction. The relaxation responses were represented as a percentage (% papaverine) of the relaxation to 100 μM papaverine. Carbachol (1 μM) generated a fast and long-duration contraction, which reached a plateau within 6 min. The PAR agonists or control peptides were added 7 min after the addition of carbachol in a non-cumulative manner, i.e., single dose administration, during the relaxation experiments. Tissues were incubated with 10 μM amastatin, an inhibitor of aminopeptidase, 15 min before beginning the experiments to reduce peptide degradation [9,20]. Amastatin did not change the tension of the muscle strip.

For studies using tetrodotoxin, the muscle strips were exposed to 1 μM tetrodotoxin for 15 min, and then to the PAR agonists. Only one concentration response, with or without tetrodotoxin, was constructed with each preparation in the experiments.

2.2. RT-PCR for detection of mRNA for PAR_1 and PAR_2 in guinea pig CBD

RT-PCR for detection of mRNA for PAR1 and PAR2 in the guinea pig CBD was performed as described previously [9,21,22] with minor modification [7]. Total RNA was isolated from the guinea pig CBD using TRIzol reagent and treated with RNAse-free DNAse I. The superscript II RNase H-reverse transcriptase system was employed for reverse transcription. Polymerase chain reaction amplification for PAR_1 was performed with Taq polymerase at 94 °C for 5 min, followed by 45 cycles at 94 °C for 30 s, 56 °C for 15 s, 72 °C for 30 s, and, finally 72 °C for 5 min. Polymerase chain reaction amplification for PAR_2 was performed with Taq polymerase at 94 °C for 15 min, followed by 35 cycles at 94 °C for 30 s, 62 °C for 1 min, 72 °C for 1 min, and, finally 72 °C for 10 min. After amplification, the PCR products were subjected to electrophoresis on a 1.5% agarose gel containing ethidium bromide and analyzed under UV light against DNA molecular markers. The following primers were used [9,21,22]:

PAR_1: Forward 5′-CCCGCTCATTTTTTCTCAGGAA-3′
Reverse 5′-AATCGGTGCCGGAGAAGT-3′
PAR_2: Forward 5′-CATGTTCAGCTACTTCCTCTCCTT-3′
Reverse 5′-AATCGGTGCCGGAGAAGT-3′

2.3. Analysis of data

Results are expressed as means ± standard error of the mean (SEM). Statistical evaluation was performed using Student's t-test or one-way analysis of variance (ANOVA), corrected by the Dunnett procedure. $P<0.05$ was considered statistically significant.

3. Results

3.1. Effects of thrombin and PAR_1 peptide agonists in CBD

In the resting guinea pig CBD strips, thrombin, 30 μM, did not cause contraction or relaxation. We then evaluated the ability of thrombin to evoke relaxation of carbachol pre-contracted CBD strips. Carbachol (1 μM) increased the force of the CBD strips by 0.36 ± 0.03 g (n = 15) and this contraction reached a plateau within 6 min (Fig. 1). Adding thrombin to the carbachol-contracted muscle strips at the plateau evoked a moderate, sustained and concentration-dependent relaxation (Figs. 1, 2). Thrombin caused detectable relaxation of the carbachol-contracted CBD strips at 3 μM and maximal relaxation at 30 μM, which produced a 41 ± 1% papaverine (100 μM)-induced relaxation (Fig. 2). Similarly, adding TFLLR-NH_2 and SFLLRN-NH_2 to the carbachol-contracted muscle strips at the plateau resulted in a moderate and concentration-dependent relaxation (Figs. 1, 2). TFLLR-NH_2 produced detectable relaxation of the carbachol-contracted strips at 10 μM and maximal relaxation at 30 μM, which produced a 38 ± 2% papaverine-induced relaxation. The highest concentration (100 μM) of TFLLR-NH_2 tested produced a 37 ± 1% papaverine-induced relaxation of carbachol-contracted CBD (Fig. 2). SFLLRN-NH_2 caused detectable relaxation of the carbachol-contracted strips at 30 μM. The highest concentration (300 μM) of SFLLRN-NH_2 tested produced a 45 ± 5% papaverine-induced relaxation of carbachol-contracted CBD (Fig. 2). In contrast, 100 μM RLLFT-NH_2, the PAR_1 inactive control peptide [23], did not cause relaxation in carbachol-contracted CBD strips (Fig. 2). The thrombin and TFLLR-NH_2-induced relaxations were not altered by

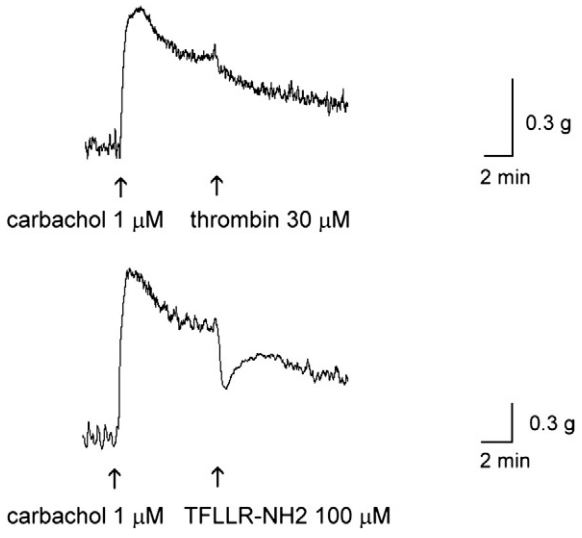

Fig. 1. Typical tracings showing the relaxation of carbachol-contracted guinea pig common bile duct with the addition of thrombin (upper panel) and the PAR_1 selective agonist TFLLR-NH_2 (Thr-Phe-Leu-Leu-Arg-NH_2, lower panel).