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Evidence that PAR-1 and PAR-2 mediate prostanoid-dependent contraction in isolated guinea-pig gallbladder

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1 We have investigated the ability of protease-activated receptor-1 (PAR-1), PAR-2, PAR-3 and PAR-4 agonists to induce contractile responses in isolated guinea-pig gallbladder. Thrombin, trypsin, mouse PAR-1 activating (SFLLRN-NH_2) peptide, and mouse PAR-2 activating (SLIGRL-NH_2) and human PAR-2 activating (SLIGKV-NH_2) peptides produced a concentration-dependent contractile response.

2 Mouse PAR-4 activating (GYPGKF-NH_2) peptide, the mouse PAR-1 reverse (NRLLFS-NH_2) peptide, the mouse PAR-2 reverse (LRGILS-NH_2) and human PAR-2 reverse (VKGILS-NH_2) peptides caused negligible contractile responses at the highest concentrations tested.

3 An additive effect was observed following the contractile response induced by either trypsin or thrombin, with the addition of a different PAR agonist (SFLLRN-NH_2 and SLIGRL-NH_2, respectively). Desensitization to PAR-2 activating peptide attenuated the response to trypsin but failed to attenuate the response to PAR-1 agonists, and conversely desensitization to PAR-1 attenuated the response to thrombin but failed to alter contractile responses to PAR-2 agonists.

4 The contractile responses produced by thrombin, trypsin, SFLLRN-NH_2 and SLIGRL-NH_2 were markedly reduced in the presence of the cyclo-oxygenase inhibitor, indomethacin, whilst the small contractile response produced by NRLLFS-NH_2 and LRGILS-NH_2 were insensitive to indomethacin.

5 The contractile responses to thrombin, trypsin, SFLLRN-NH_2 and SLIGRL-NH_2 were unaffected by the presence of: the non-selective muscarinic antagonist, atropine; the nitric oxide synthase inhibitor, L-NAME; the sodium channel blocker, tetrodotoxin; the combination of selective tachykinin NK_1 and NK_2 receptor antagonists, (S)-1-[2-[3-(3,4-dichlorphenyl)-1 (3-isopropoxyphenylacetyl) piperidin-3-yl] ethyl]-4-phenyl-1 azaniabicyclo [2.2.2] octane chloride (SR140333) and (S)-N-methyl-N-[4-acetylamino-4-phenylpiperidino-2-(3,4-dichlorophenyl)-butyl] benzamide (SR48968), respectively.

6 The results indicate that PAR-1 and PAR-2 activation causes contractile responses in the guinea-pig gallbladder, an effect that is mediated principally by prostanoid release, and is independent of neural mechanisms.

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Keywords: Gallbladder; guinea-pig; protease-activating receptor; prostanoids; thrombin; trypsin

Abbreviations: CCh, carbachol; DMSO, dimethylsulphoxide; Indo, indomethacin; L-NAME, N^{ω}-nitro-L-arginine methyl ester; NK, neurokinin; NO, nitric oxide; PAR, protease-activated receptor

Introduction

The first receptor of a new expanding subclass of G protein coupled receptors, protease-activated receptor-1 (PAR-1), was cloned in the early 1990s (Vu *et al*., 1991). This was subsequently followed by the cloning of PAR-2 (Nystedt *et al*., 1994), PAR-3 (Ishihara *et al*., 1997) and PAR-4 (Xu *et al*., 1998). The unique feature of PARs is that they are activated by proteolysis (Ishihara *et al*., 1997; Vu *et al*., 1991). Proteases cleave the PARs within the extracellular N-terminus domain to expose a new N-terminus that acts as a tethered ligand by binding to extracellular domains of the receptor, and thereby activating the cleaved receptor molecule (Dery *et al*., 1998; Hollenberg, 1996; Nystedt *et al*., 1994; Vu *et al*., 1991). Thrombin preferentially cleaves PAR-1 (Vu *et al*., 1991), PAR-3 (Ishihara *et al*., 1997) and PAR-4 (Xu *et al*., 1998). Tethered ligands for PAR-1 (mouse SFLLRN-NH_2; Vu *et al*., 1991) and PAR-4 (mouse GYPGKF-NH_2; Xu *et al*., 1998) also activate the corresponding PAR without receptor cleavage (Hollenberg, 1996; Xu *et al*., 1998), whilst the PAR-3 activating peptide does not appear to stimulate its receptor (Lan *et al*., 2000). Mouse PAR-2 was first cloned by a reduced stringency hybridization search of a genomic library with a tachykinin NK_2 receptor-derived oligonucleotide (Nystedt *et al*., 1994). The synthetic peptide corresponding to the tethered ligand domain (mouse SLIGRL-NH_2 and human SLIGKV-NH_2) is able to activate the receptor. However, both PAR-1 and PAR-2 activating peptides are usually about 2–3 orders of magnitude less potent than proteases (Hollenberg, 1996; Saifeddine *et al*., 1996).

Both PAR-1 and PAR-2 activation leads to relaxant responses in vascular tissue (Cheung *et al*., 1998; Hamilton *et al*., 1998; Hollenberg, 1996). However, in extravascular tissue PAR-1 and PAR-2 activation has been demonstrated to induce

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both relaxant (Cocks *et al*., 1999a, b; Corvera *et al*., 1997) and contractile responses (Kawabata *et al*., 1999; Lan *et al*., 2000; Saifeddine *et al*., 1996). A number of studies have looked at the contractile properties of the gallbladder, however there is no information at present on the role of thrombin, trypsin and PARs in this tissue. Recently, it has been demonstrated that there is an increase in the level of trypsin in patients with stones in the bile duct (Vracko & Wiechel, 2000). Therefore, it is of interest to investigate the physiological role of these receptors in the gallbladder. Here, we have investigated whether PAR-1, PAR-2, PAR-3 and PAR-4 may produce motor responses in guinea-pig isolated gallbladder. We found that thrombin, trypsin, SFLLRN-NH_2, SLIGRL-NH_2 and SLIGKV-NH_2 caused a concentration-dependent contraction, and so the mechanism of this contractile response was also studied.

Methods

Male albino guinea-pigs (200–300 g) were anaesthetized with ether and decapitated (all experiments complied with the national guidelines and were approved by the regional ethical committee). The gallbladders were carefully dissected from the surrounding tissue and cut in half horizontally from the proximal to distal region. The two halves were tied at each end and placed in 5 ml organ baths containing Krebs buffer solution ((mM): NaCl 119, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.5, $CaCl_2$ 2.5, KCl 4.7 and glucose 11) maintained at 37°C and oxygenated (96% O_2 and 4% CO_2), at a tension of 0.75 g. The gallbladder halves were connected to an isometric force transducer (Ugo Basile, Unirecord 7050) for recording the mechanical activity. During the initial stabilization period (90 min), tissues were washed every 20 min. In all tissues, carbachol (1 μM) was administered to ascertain the sensitivity of the tissue prior to the beginning of the experiment. Due to the tissue possessing spontaneous phasic contractions, measurements for the contractile or relaxant response to selective agonists were taken from the mean spontaneous activity value obtained at the plateau of each response. The data are expressed as a percentage of the contractile response obtained by carbachol (1 μM).

Cumulative concentration response curves to thrombin (0.01–10 μM), trypsin (0.1–100 nM); mouse PAR-1 (SFLLRN-NH_2), PAR-2 (SLIGRL-NH_2) and PAR-4 (GYPGKF-NH_2), and human PAR-2 (SLIGKV-NH_2) activating peptides (10 nM–100 μM); mouse PAR-1 (NRLLFS-NH_2) and PAR-2 (LRGILS-NH_2), and human PAR-2 (VKGILS-NH_2) reverse peptides (10 nM–100 μM) were performed. In another set of tissues, cumulative response curves to trypsin or thrombin were performed, after the final concentration had plateaued either SFLLRN-NH_2 or SLIGRL-NH_2 (100 μM) were added. The tissues were allowed to equilibrate for 1 h between each cumulative concentration response curve.

In tissues pre-contracted with the thromboxane receptor agonist, U46619-8 (10 nM), cumulative concentration response curves were performed in the absence or presence of thrombin (0.01–10 μM) or trypsin (0.1–100 nM). In another set of experiments, desensitization to either SLIGRL-NH_2 or SFLLRN-NH_2 was performed, by repeatedly performing cumulative concentration response curves over three consecutive periods (20 min interval between each curve without washing). Contractile responses to SFLLRN-NH_2 were tested in SLIGRL-NH_2 desensitized tissues, and contractile responses to SLIGRL-NH_2 were tested in SFLLRN-NH_2 desensitized tissues. In another set of experiments desensitisation was performed with either SLIGRL-NH_2 or SFLLRN-NH_2. Then cumulative concentration response curves to both trypsin and thrombin were performed.

The effect of thrombin, trypsin, SFLLRN-NH_2 and SLIGRL-NH_2 were studied either in the absence or presence of indomethacin (5 μM), atropine (1 μM), N^{ω}-nitro-L-arginine methyl ester (L-NAME: 100 μM) and tetrodotoxin (0.3 μM). In another set of experiments, cumulative concentration curves to thrombin, trypsin, SFLLRN-NH_2, SLIGRL-NH_2 and substance P were performed in the absence or presence of a combination of the selective tachykinin NK_1 and NK_2 receptor antagonists, (S)-1-[2-[3-(3,4-dichlorphenyl)-1 (3-isopropoxyphenylacetyl) piperidin-3-yl] ethyl]-4-phenyl-1 azaniabicyclo [2.2.2] octane chloride (SR140333: 0.1 μM) and (S)-N-methyl-N-[4-acetylamino -4- phenylpiperidino-2- (3,4-dichlorophenyl)-butyl] benzamide (SR48968: 0.1 μM), respectively. Tissues were incubated for 15 min with the various treatments, except for

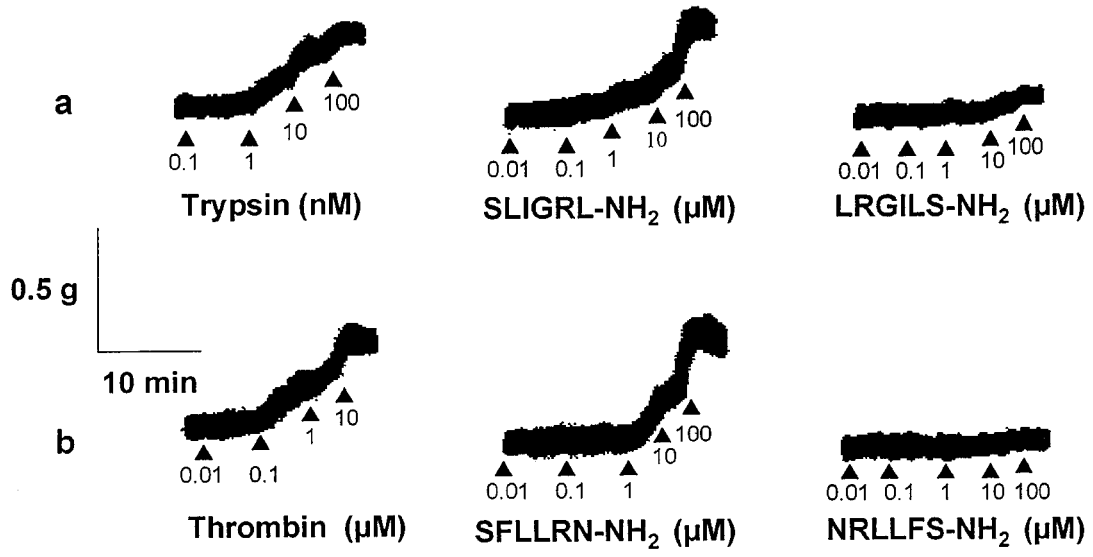

Figure 1 Typical tracing representing the contractile response to (a) trypsin, mouse PAR-2 activating (SLIGRL-NH_2) and PAR-2 reverse (LRGILS-NH_2) peptides; (b) thrombin, mouse PAR-1 activating (SFLLRN-NH_2) and PAR-1 reverse (NRLLFS-NH_2) peptides in isolated guinea-pig gallbladder. Representative traces of a minimum of six experiments.

indomethacin, which was incubated for 45 min prior to the cumulative concentration response curves to the agonists.

Materials

SFLLRN-NH_2, NRLLFS-NH_2, SLIGRL-NH_2, LRGILS-NH_2, SLIGKV-NH_2, VKGILS-NH_2 and GYPGKF-NH_2 were synthesized in the Department of Pharmaceutical Sciences of the University of Ferrara (Italy). Trypsin was from Worthington Biochemical Co. (Freehold, NJ, U.S.A.). SR140333 and SR48968 were a gift from Dr X. Emonds-Alt (Sanofi Recherché, Montpellier, France). Atropine, indomethacin, L-NAME, substance P, tetrodotoxin, thrombin and U46619-8 were from Sigma (Italy). Agents were dissolved in Krebs buffer, except for indomethacin, SR140333 and SR48968, which at stock concentrations (10, 1 and 1 mM, respectively) were dissolved in 100% DMSO.

Statistical analysis

Results are expressed as mean ± s.e.mean. Statistical analysis was performed by means of Student's *t*-test, analysis of variance (ANOVA) and Dunnett's test when required. If $P<0.05$ the results were considered significant.

Results

Under the present experimental conditions guinea-pig gallbladder smooth muscle showed spontaneous phasic contractions that can clearly be observed on the traces (Figures 1 and 3). Thus, the results are expressed as the mean spontaneous activity. In preliminary experiments we found that concentration related responses, obtained at the first concentration response curve to the various PAR activating peptides, were significantly lower than those obtained at the second curve. However, the third and fourth curves were not significantly different from the second curve ($P<0.05$, $n=8$). The data presented in this paper are those obtained at the second curve, that were compared to those obtained at the third curve in the presence of various drug treatments.

In guinea-pig isolated gallbladder thrombin (10 μM), trypsin (0.1 μM), mouse PAR-1 (SFLLRN-NH_2: 100 μM) and PAR-2 (SLIGRL-NH_2: 100 μM), and the human PAR-2 (SLIGKV-NH_2: 100 μM) activating peptides at the maximal concentration (due to peptide availability and presumed protease selectivity) produced a contractile response (% carbachol 1 μM) of 56 ± 5.0% ($n=9$), 39 ± 8.0% ($n=12$), 70 ± 4.0% ($n=8$), 45.0 ± 8.0% ($n=10$) and 37 ± 6.0% ($n=12$), respectively (Figures 1 and 2). PAR activating peptides produced a rapid onset contractile response, which was sustained for approximately 5 min. The threshold concentration that produced a measurable response for thrombin, trypsin, SFLLRN-NH_2, SLIGRL-NH_2 and SLIGKV-NH_2 was 0.1 μM, 1 nM, 1 μM, 0.1 μM and 1 μM, respectively. Addition of SFLLRN-NH_2 (100 μM) on top of the maximum response to trypsin (0.1 – 100 nM) produced a greater contractile response (49 ± 4%) than that produced by trypsin alone (37 ± 5%; $P<0.05$, $n=6$). In contrast, addition of SLIGRL-NH_2 (100 μM) on top of the maximum response produced by trypsin (38 ± 4%) was similar to the response produced by trypsin alone (36 ± 5%; $P>0.05$, $n=6$). Conversely, addition of SLIGRL-NH_2 (100 μM) on top of the maximum response to thrombin (0.01 – 10 μM) produced a greater contractile response (68 ± 4%) than that produced by thrombin alone (54 ± 5%; $P<0.05$, $n=6$). Finally, addition of SFLLRN-NH_2 (100 μM) on top of the maximum response produced by thrombin (56 ± 4%) was similar to the response produced by thrombin alone (57 ± 6%; $P>0.05$, $n=6$).

Mouse PAR-1 (NRLLFS-NH_2) and PAR-2 (LRGILS-NH_2), and the human PAR-2 (VKGILS-NH_2: $P>0.05$, $n=7$) reverse peptides only produced a slight contractile response at the highest concentration (100 μM) tested (Figures 1 and 2). In other experiments, the mouse PAR-4 activating peptide (GYPGKF-NH_2), produced only negligible contractile response (Figure 2b). In tissues pre-contracted with U46619-8 (10 nM), thrombin (0.01 – 10 μM) and trypsin (0.1 – 100 nM) failed to induce relaxation ($P>0.05$, $n=6$).

In the desensitization studies the first and third cumulative concentration response curves produced a maximal response

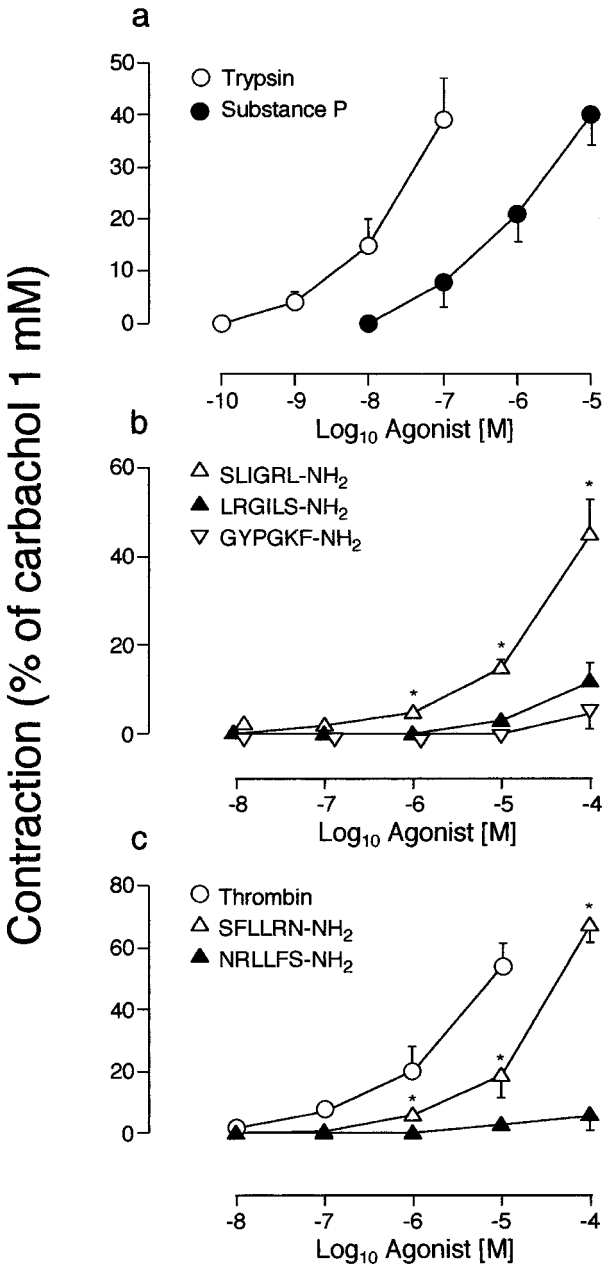

Figure 2 Cumulative concentration response curves to (a) trypsin and substance P; (b) mouse PAR-2 activating (SLIGRL-NH_2), PAR-2 reverse (LRGILS-NH_2) and PAR-4 activating (GYPGKF-NH_2) peptides; (c) thrombin, mouse PAR-1 activating (SFLLRN-NH_2) and reverse (NRLLFS-NH_2) peptides in isolated guinea-pig gallbladder. Each point represents mean ± s.e.mean of at least six experiments. *$P<0.05$ vs reverse peptides LRGILS-NH_2 and NRLLFS-NH_2.

(% carbachol 1 μM) to SLIGRL-NH_2 (100 μM) of 47±6 and 10±4%, respectively. Following SLIGRL-NH_2 desensitisation the contractile response to SFLLRN-NH_2 (100 μM) was 65±8% ($P>0.05$ compared with control, $n=6$; Figure 3a). Conversely, in SFLLRN-NH_2 desensitized tissue, the first and third cumulative concentration response curves produced a maximal response to SFLLRN-NH_2 (100 μM) of 69±8.0 and 15±4.0%, respectively. Following SFLLRN-NH_2 desensitization the contractile response to SLIGRL-NH_2 was 52±8% ($P>0.05$ compared with control, $n=6$; Figure 3b). In another set of experiments three repeat cumulative concentration response curves to either SLIGRL-NH_2 or SFLLRN-NH_2 were performed as before ($P<0.05$, $n=6$). Desensitization to SLIGRL-NH_2 also desensitized the tissue to trypsin (0.1 μM) (control, 38±6% vs desensitized, 6±4%; $P<0.05$, $n=6$), and conversely desensitization to SFLLRN-NH_2 attenuated the response to thrombin (10 μM) (control, 57±5% vs desensitized, 10±4%; $P<0.05$, $n=6$). However, desensitization with the PAR-2 agonists had no effect on PAR-1 (thrombin 10 μM) (control, 57±4% vs desensitized, 59±6%; $P<0.05$, $n=6$), and conversely desensitization with PAR-1, agonists had no effect on PAR-2 (trypsin 0.1 μM) (control, 38±9% vs desensitized, 40±7%; $P<0.05$, $n=6$).

In the presence of indomethacin (5 μM) the contractile response to thrombin, trypsin, SFLLRN-NH_2 and SLIGRL-NH_2 was practically abolished (Figure 4 and Table 1). In contrast, the small contractile response produced by NRLLFS-NH_2 and LRGILS-NH_2 (100 μM) was not reduced by the presence of indomethacin ($P>0.05$, $n=7-9$). The contractile response of thrombin, trypsin, SFLLRN-NH_2 and SLIGRL-NH_2 was insensitive to atropine (1 μM), L-NAME (100 μM) and tetrodotoxin (0.3 μM). Furthermore, the combination of SR140333 and SR48968 (0.1 μM) had no effect on the contractile response to thrombin, trypsin, SFLLRN-NH_2 or SLIGRL-NH_2 (Table 1). This is in contrast to the almost complete attenuation of the substance P contractile response (% carbachol 1 μM: 54±8%; $n=6$) in the presence of the tachykinin receptor antagonists (% carbachol 1 μM: 5±4%; $P<0.05$, $n=6$).

Discussion

We have shown in this study that the activation of PAR-2 and PAR-1 produces a contractile response in guinea-pig gallbladder smooth muscle *in vitro*. Trypsin is known to activate PAR-2 and PAR-4. Thus, its ability to stimulate contraction of guinea-pig gallbladder at low (nM) concentrations suggests that PAR-2 and/or PAR-4 mediate this effect. Evidence for the involvement of PAR-2 in the contractile response to trypsin has been demonstrated with the use of PAR-2 activating (SLIGRL-NH_2) peptide, which caused a remarkable contractile response. Furthermore, the mouse PAR-2 (LRGILS-NH_2) and the human PAR-2 (VKGILS-NH_2) reverse peptides showed only minor activity at the highest concentration tested (100 μM). Thus, the amino-acid sequence required to cause contraction of the guinea-pig gallbladder corresponds to the tethered ligand contained in PAR-2. Finally, a role for PAR-4 has been excluded by the finding that the PAR-4 activating (GYPGKF-NH_2) peptide was without effect in this tissue preparation.

Both thrombin and the PAR-1 activating (SFLLRN-NH_2) peptide produced a concentration dependent response, thus suggesting that PAR-1 activation mediates the response to thrombin. However, it has been suggested that the PAR-1 activating peptide is capable of activating PAR-2 (Blackhart *et al*., 1996; Hollenberg *et al*., 1997). Therefore, it is feasible to hypothesize that the PAR-1 tethered ligand could be activating either PAR-2 or both PAR-1 and PAR-2 in this tissue. However, our findings would suggest that this is not the case as cumulative concentration response curves performed with trypsin or thrombin showed additional contractile responses

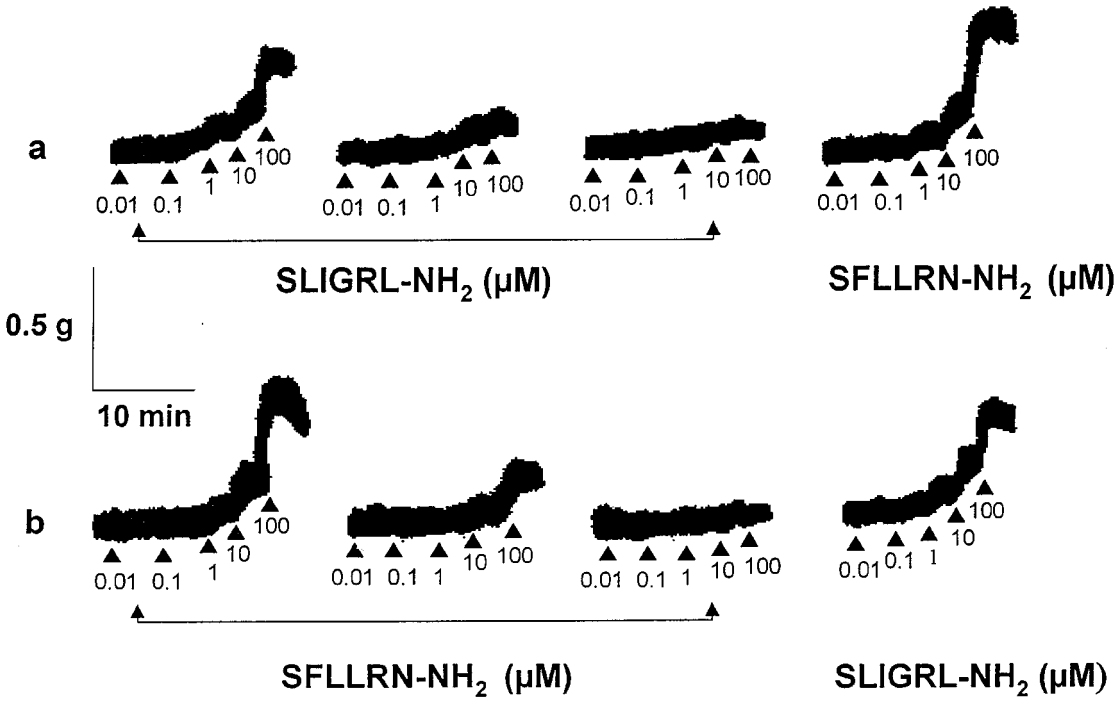

Figure 3 Typical traces representing the effect of (a) desensitization to mouse PAR-2 (SLIGRL-NH_2) activating peptide on PAR-1 (SFLLRN-NH_2) activating peptide contractile responses, and (b) desensitization to PAR-1 (SFLLRN-NH_2) activating peptide on PAR-2 (SLIGRL-NH_2) activating peptide contractile responses in isolated guinea-pig gallbladder. Representative traces of a minimum of six experiments.

after the addition of a different PAR agonist. Desensitisation studies further substantiate these findings as SLIGRL-NH_2 desensitization failed to attenuate the response to SFLLRN-NH_2 and thrombin, and conversely SFLLRN-NH_2 desensitization failed to attenuate SLIGRL-NH_2 and trypsin contractile responses. These findings imply that thrombin and the PAR-1 activating peptide induce contractile responses *via* a PAR-1-mediated mechanism. However, because a selective PAR-3 activating peptide is not available we can not exclude that PAR-3 may, at least in part, contribute to the contractile response induced by thrombin in this tissue.

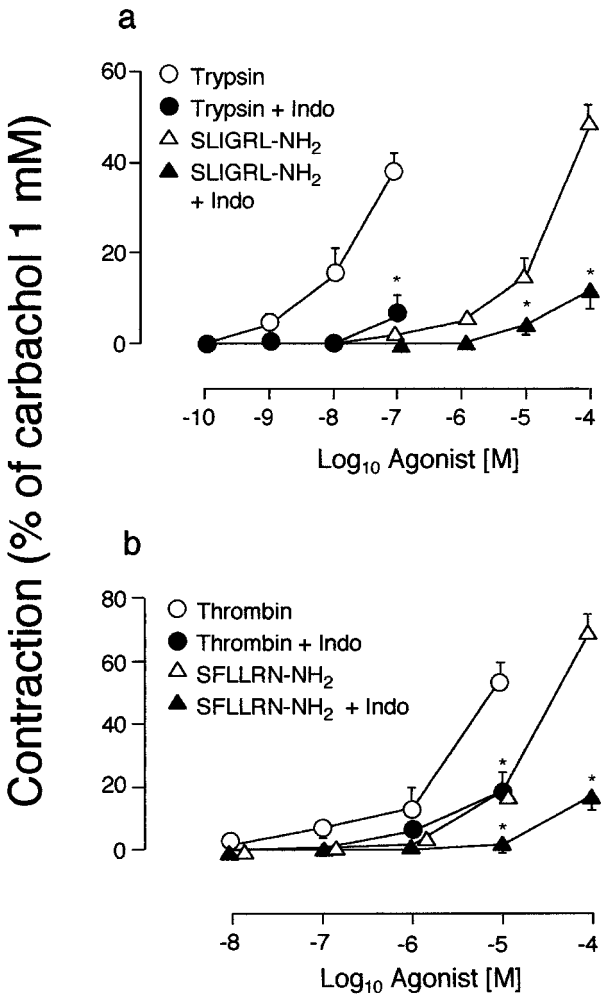

Figure 4 The contractile response to (a) trypsin and mouse PAR-1 (SLIGRL-NH_2) activating peptide, (b) thrombin and mouse PAR-1 (SFLLRN-NH_2) activating peptide in the absence or presence of indomethacin (Indo: 5 μM) in isolated guinea-pig gallbladder. Each bar represents mean + s.e.mean of at least six experiments. $^*P<0.05$ vs vehicle controls.

In previous studies, thrombin and PAR-1 activating peptides produced a biphasic contractile and relaxant response in the airways, gastric and duodenal longitudinal smooth muscle (Cocks *et al*., 1999a, b; Hollenberg *et al*., 1993; Kawabata *et al*., 1999; Lan *et al*., 2000). However, we have excluded the role of PAR-1 or PAR-2 activation in mediating relaxation, as both thrombin and trypsin failed to induce relaxation in U46619-8 pre-contracted tissue. In agreement with a previous study (Kawabata *et al*., 1999) we have ruled out the role of nitric oxide (NO) in the guinea-pig gallbladder, as L-NAME failed to alter the contractile response to PAR-1 and PAR-2 activation. Furthermore, as with previous observations (Hollenberg *et al*., 1993; Saifeddine *et al*., 1996), we have demonstrated that indomethacin caused an almost complete attenuation of the contractile response induced by thrombin, trypsin, SFLLRN-NH_2 and SLIGRL-NH_2. The indomethacin-resistant small contraction observed at the highest concentration of SLIGRL-NH_2 (100 μM) was similar to the contractile response induced by the indomethacin-insensitive response of LRGILS-NH_2 at the same concentration. Therefore, it is possible to hypothesize that this effect is a non-specific and non-receptor mediated response due to elevated concentrations of these molecules.

Acetylcholine released from postganglionic cholinergic nerves *via* muscarinic receptor activation, or tachykinins released from terminals of primary sensory neurons and activation of NK_2 and NK_1 receptors (Patacchini *et al*., 1997) are the main neural pathways that cause contraction of the guinea-pig gallbladder. It has recently been reported that PAR-2 activation stimulates the release of substance P from terminals of capsaicin-sensitive primary sensory neurons, thus causing neurogenic inflammatory responses in peripheral tissue (Steinhoff *et al*., 2000). However, experiments with atropine or with SR140333 and SR48968 (which abolished the substance P mediated response: Croci *et al*., 1995), suggest that the contractile response produced by PAR-1 and PAR-2 agonists are not mediated *via* either acetylcholine or tachykinins such as substance P. Furthermore, experiments with tetrodotoxin, an

Table 1 The contractile response seen in isolated guinea-pig gallbladder to various PAR agonists in the presence of selective antagonists or inhibitors

	Thrombin 10 μM (% CCh 1 mM)	*SFLLRN-NH_2* 100 μM (% CCh 1 mM)	*Tryspin* 0.1 μM (% CCh 1 mM)	*SLIGRL-NH_2* 100 μM (% CCh 1 mM)
Vehicle	56±5	70±4	39±8	45±8
	$n=9$	$n=8$	$n=12$	$n=10$
Indomethacin (5 μM)	19±5*	18±6*	6±2*	12±3*
	$n=7$	$n=6$	$n=10$	$n=8$
Atropine (1 μM)	45±9	67±9	41±7	46±8
	$n=6$	$n=6$	$n=8$	$n=6$
L-NAME (100 μM)	43±6	59±7	46±8	45±10
	$n=6$	$n=6$	$n=8$	$n=6$
Tetrodotoxin (0.3 μM)	46±9	66±4	45±6	45±9
	$n=6$	$n=6$	$n=8$	$n=6$
SR140333/SR48968 (0.1 μM)	41±9	61±7	39±2	41±10
	$n=6$	$n=6$	$n=6$	$n=6$

Each figure represents the mean±s.e.mean of the contractile response of the PAR agonist in the absence or presence of the selective antagonists/inhibitors. The figures represent a percentage of the Emax carbachol (1 μM) response. $^*P<0.05$ vs vehicle control.

inhibitor of fast sodium channels, also indicate that neural mechanisms are not involved.

The common bile duct terminates in the ampulla where the pancreatic duct also terminates. If the efflux of the secretions of these ducts is blocked at the level of the sphincter of Oddi, trypsin contained in pancreatic secretion might diffuse back into the gallbladder causing contraction of this organ. In pathophysiological conditions such as stones in the gallbladder and bile duct, there is evidence of elevated levels of trypsin (Vracko & Wiechel, 2000). Furthermore, a number of clinical studies have shown the beneficial effect of non-steroidal anti-inflammatory drugs in reducing biliary colic pain (Alwaili & Saloom, 1998; Akriviadis *et al*., 1997). Plasma thrombin may extravasate from the vascular lumen into the gallbladder wall during inflammation. With this in mind it might be of interest to investigate whether PAR-1 and PAR-2 *via* prostanoid activation may exert any motor effects in the human gallbladder.

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