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# Activation of mast cells induced by agonists of proteinase-activated receptors under normal conditions and during acute inflammation in rats

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**Abstract**

Functions of thrombin as a modulator of inflammation and tissue repair are mediated by the proteinase-activated receptor (PAR) family. Some of these effects may be induced by activation of mast cells. To characterize the degranulation of rat peritoneal mast cells in response to PAR agonists, the effects of thrombin, trypsin and peptide agonists of PARs (PAR-AP, proteinase-activated receptor-activating peptides) on secretion were investigated. The release of β-hexosaminidase by thrombin (0.01–1 μM) was concentration-dependent and mediated via $PAR_1$, as evidenced by cathepsin G (100 μM)-induced inactivation of $PAR_1$ and thrombin-stimulated $PAR_1$ desensitization. Trypsin (1 μM) accelerated histamine secretion. The $PAR_1$-AP, TRAP (SFFLRN, 1–100 μM) and the $PAR_2$-AP SLIGRL (5–100 μM) caused the release of histamine, and β-hexosaminidase from inflammatory mast cells were obtained from a model of acute peritonitis in rats. Relative to the response to compound 48/80, the thrombin- and TRAP-induced release of β-hexosaminidase was higher in inflammatory mast cells than in the control. This suggests that additional exposure of $PAR_1$ on mast cells to PAR agonists or an increase in PARs sensitivity to PAR agonists probably occurred during acute inflammation.


*Keywords:* Mast cell; PAR (proteinase-activated receptor); PAR-activating peptide (PAR-AP); Thrombin; Histamine; Peritonitis

## 1. Introduction

Blood coagulation and thrombin generation occur at sites of vascular injury, inflammation and wound healing (Cocks and Moffatt, 2000; Strukova 2001; Strukova et al., 1996; Vergnolle et al., 2001). Thrombin stimulates various cells involved in inflammation (e.g. monocytes, lymphocytes, neutrophils, fibroblasts and mast cells) to release mediators such as histamine, cytokines or eicosanoids (Brass, 1997; Coughlin, 1999; Dery et al., 1998; Strukova et al., 1996). The proinflammatory effects of thrombin on cells are mediated by membrane-bound protease-activated receptors (PARs). Four members of the PAR family have been recognized: $PAR_1$, $PAR_3$ and $PAR_4$ are receptors for thrombin, and $PAR_2$ is a receptor for trypsin, tryptase and factor Xa (Camerer et al., 2000; Coughlin, 1999; Dery et al., 1998). Some proteases cleave PARs at sites other than the activation site to initiate signaling; thereafter, the receptor is unresponsive to subsequent proteolytic activation. Thus, in addition to the N-terminal $Arg^{41}$–$Ser^{42}$ site cleaved by thrombin, cathepsin G, a serine protease released by activated neutrophils, cleaves $PAR_1$ at $Phe^{43}$–$Leu^{44}$ and $Phe^{55}$–$Trp^{56}$, removing the tethered ligand and rendering the receptor unresponsive to thrombin (Molino et al., 1995).

Short, synthetic peptides corresponding to the amino acid sequence of the tethered ligand newly generated after proteolytic cleavage of PARs can selectively activate PARs (proteinase-activated receptor-activating peptides, PAR-AP) and mimic the effects of proteases.

Mast cells are actively involved in inflammatory and repair processes since they release a wide range of proinflammatory, vasoactive mediators, cytokines and growth factors in response to activation by immune and nonimmune liberators (Galli, 2000; Metcalfe et al., 1997). Certain proteinases, including thrombin and factor Xa, are involved in inflammation and tissue repair, and can come into contact with mast cells, which are localized in almost all tissues and along blood and lymphatic vessels at sites of tissue damage (Razin and Marx, 1984; Razin et al., 1985; Strukova et al.,

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1996). It was suggested that PAR activation at inflammatory sites may be associated with mast cell degranulation (Cirino et al., 1996; Strukova, 2001; Vergnolle et al., 2001). It is not quite clear whether thrombin and thrombin-like proteinases directly induce mast cell degranulation or whether this process may be mediated by the effects of thrombin on other cells that in turn release substances able to promote mast cell degranulation. The direct interaction of mast cells and serine proteinases has been suggested by detection of $PAR_1$- and $PAR_2$-mRNA in these cells and by the fact that thrombin accelerates mast cell secretion (D'Andrea et al., 2000; Nishikawa et al., 2000; Stenton et al., 2002; Strukova et al., 1996, 1999; Umarova et al.,2000).

In the present study, we investigated the ability of thrombin, trypsin and PAR-APs to induce activation and release of mediators from rat peritoneal mast cells. The release of histamine and β-hexosaminidase was measured. Furthermore, we studied the mast cell-PAR system in rats after producing acute peritonitis by intraperitoneal injection of thioglycolate.

## 2. Materials and methods

### 2.1. Animal studies

Rats (male Wistar rats, 300–350 g) had free access to food and water, and were housed under constant temperature (22 °C) and photoperiod (12-h light–dark cycle). All experimental procedures were approved by the Animal Care Committee of the Moscow State University. Crude peritoneal mast cells were isolated via lavage of the peritoneal cavity of anesthetized rats (sodium pentobarbital, 40 mg/kg, i.p.) as described previously (Strukova et al., 1996).

Acute inflammation in vivo was induced by intraperitoneal injection of thioglycolate broth (2 ml 40% w/v) as described previously (Melnicoff et al., 1989; Pejler, 1999). Mast cells were isolated and examined after 16 h.

### 2.2. Experiments with isolated mast cells

Mast cells were purified by centrifugation on Ficoll density gradients (30% and 40%) and washed three times in Tyrode's solution containing 10 mM HEPES, 145 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM glucose and 0.1% bovine serum albumin, pH 7.2. The resulting cell suspension was diluted to the concentration required and kept on ice. For the experiments, 900-μl cell suspension containing $5 \times 10^5$ cells was preincubated at 37 °C for 5 min. Thereafter, 100 μl of agonists (thrombin, $PAR_1$-APs, $PAR_2$-AP and others) was added to the cell suspensions. After a period of 10 min, the incubation was stopped by immersion in cold Tyrode's solution (3 ml, pH 7.4). After centrifugation at $400 \times g$ for 10 min, the supernatants were collected for histamine and β-hexosaminidase determination. The pellets were resuspended in 4 ml of Tyrode's solution, and cells were destroyed by addition of 0.5 ml 0.1% Triton X-100 or boiling for 5 min. After centrifugation at $400 \times g$ for 10 min, the supernatants were assayed for histamine and β-hexosaminidase. In some experiments, only the release of β-hexosaminidase was determined because there was interference from exogenous peptides when histamine was measured fluorimetrically.

### 2.3. Assays for the release of mediators

Histamine was measured spectrofluorometrically both in the pellet (residual histamine) and in the supernatant (released histamine) according to Shore's (1971) method. The results are expressed as the percentage of histamine release related to the total histamine content. β-Hexosaminidase activity was determined using the chromogenic substrate *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide (Schwartz et al., 1982). The percentage of release was calculated from the following formula: release (%)=(stimulated release − unstimulated release)/(stimulated release +residual) × 100%.

### 2.4. Materials

Bovine α-thrombin (2500 NIH U/mg) was purified from commercial preparations as decribed in Strukova et al. (1996); compound 48/80 and trypsin were from Serva (Heidelberg, Germany); cathepsin G was from Sigma (St. Louis, MO, USA); thioglycolate was from Fluka (Buchs, Switzerland). TRAP (thrombin receptor-activating peptide: SFFLRN, murine), FLLRN, control peptide FLLRN, haTRAP (high affinity: AF(fluoro)R(Cha)RY-$NH_2$ where Cha represents cyclohexyl-Ala) and $PAR_2$-AP (SLIGRL, murine) were from Biosyntan (Berlin, Germany). Mpr(Cha) (Mercaptopropionyl-F(Cha-Cha)RKPNDK-$NH_2$) was kindly provided by Dr. A. Kawabata and *O*-phthaldialdehyde by Dr. G. Pejler.

### 2.5. Statistical analysis

Statistical analysis was performed using the Student's unpaired *t*-test. Data are presented as means ± S.E.M. from five to nine independent experiments. The differences were considered to be statistically significant at $P < 0.05$.

## 3. Results

### 3.1. Thrombin-induced mast cell activation

In previous experiments, it was found that α-thrombin (0.1–1.0 μM) was able to increase the secretion of histamine (Strukova et al., 1996). In these studies, it was shown that α-thrombin (0.01–1 μM) also induced an increase in β-hexosaminidase release from rat peritoneal mast cells (Fig. 1). This effect of thrombin might be due to proteolytic

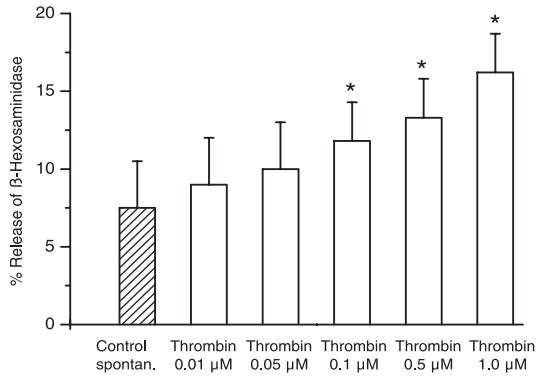


Fig. 1. Concentration–response relationship for the thrombin-induced release of β-hexosaminidase from rat peritoneal mast cells. Control represents spontaneous release. Data are means ± S.E.M. from five separate experiments. *$P<0.05$ compared to control.

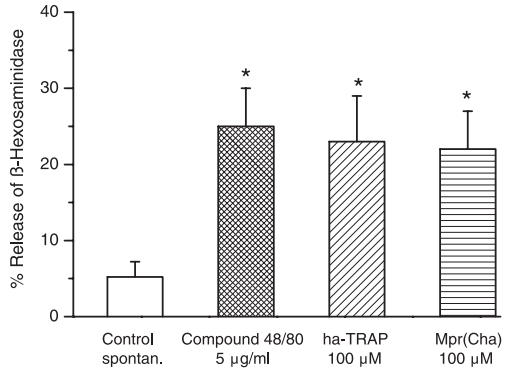


Fig. 3. Release of β-hexosaminidase from rat peritoneal mast cells induced by compound 48/80, haTRAP and Mpr(Cha). Control represents spontaneous release. Data are means ± S.E.M. from four to five separate experiments. *$P<0.05$ compared to control.

activation of mast cell PARs. To investigate this possibility, the effect of the $PAR_1$-AP, TRAP (SFFLRN), on histamine and β-hexosaminidase secretion from mast cells was investigated. As demonstrated in Fig. 2, TRAP (1–100 μM) caused a concentration-dependent increase in the release of the mediator. Maximum release of histamine (41.6 ± 3.5 %) and β-hexosaminidase (14.1 ± 2.9%) was reached at 50 μM TRAP, and at 100 μM their release was reduced. Another $PAR_1$-AP, haTRAP, which has high affinity for platelets and endothelial cells (Feng et al., 1995), caused marked secretion of β-hexosaminidase (Fig. 3). Control peptide, FLLRN (1.0–100 μM), had no the effect on resting β-hexosaminidase level (data not shown). To antagonize the $PAR_1$-AP-induced activation of mast cells, we used the peptide Mpr(Cha), which is a $PAR_1$ antagonist on platelets and human embryonic kidney cells, but a full agonist for $PAR_2$ on human embryonic kidney cells (Kawabata et al., 1999). Indeed, almost complete inhibition of haTRAP-induced platelet aggregation after pretreatment of platelets with Mpr(Cha) was observed (not shown). However, Mpr (Cha) produced strong degranulation of mast cells with a potency similar to that observed for haTRAP and the polybasic secretagogue compound 48/80 (5 μg/ml) (Fig. 3).

Cathepsin G is known to hydrolyze the $Phe^{43}$–$Leu^{44}$ and $Phe^{55}$–$Trp^{56}$ bond of peptides, as well as the $Arg^{41}$–$Ser^{42}$ bond in the N-terminus of $PAR_1$ (Molino et al., 1995). The cleavage of N-terminal peptide bonds results in desensitization of $PAR_1$ to thrombin. Preincubation of mast cells with cathepsin G (100 μM) reduced the thrombin (0.5 μM)-induced secretion of mediators to the basal level of secretion, consistent with $PAR_1$ being the target receptor of thrombin on mast cells.

### 3.2. The effect of PAR2-AP on mast cell activation

The $PAR_2$-AP, SLIGRL (5–100 μM), caused a concentration-dependent elevation of mediator release from mast cells (Fig. 4). The release of histamine was more pronounced than that of β-hexosaminidase. Furthermore, we studied the response of mast cells to trypsin, an agonist for $PAR_2$. Trypsin at a concentration of 1μM enhanced histamine secretion by 19.4 ± 6.1% ($P<0.05$, $n=4$). These

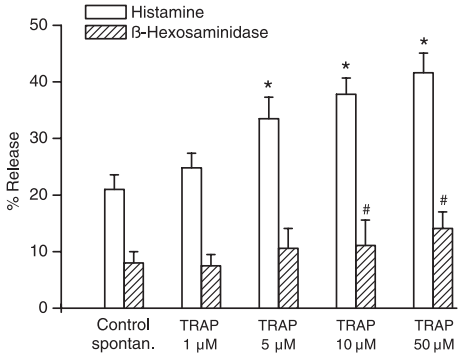


Fig. 2. Concentration–response relationship for the TRAP (SFFLRN)-induced release of histamine and β-hexosaminidase from rat peritoneal mast cells. Controls represent spontaneous release. Data are means ± S.E.M. from five separate experiments. *$P<0.05$ versus control histamine, #$P<0.05$ versus control β-hexosaminidase.

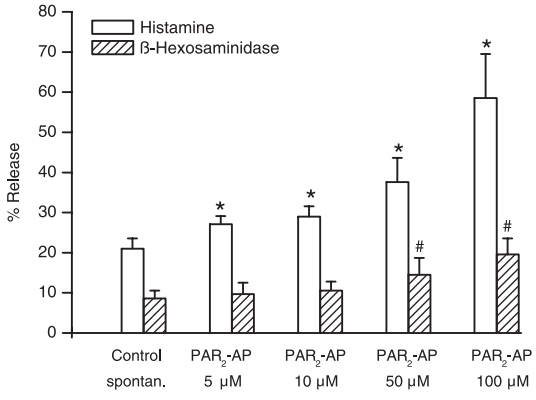


Fig. 4. Concentration–response relationship for the $PAR_2$-AP (SLIGRL)-induced release of histamine and β-hexosaminidase from rat peritoneal mast cells. Control represents spontaneous release. Data are means ± S.E.M. from five separate experiments. *$P<0.05$ compared to control to histamine, #$P<0.05$ compared to control to β-hexosaminidase.

results suggest that in mast cells, $PAR_2$ activation might contribute to cell degranulation and mediator release.

### 3.3. Thrombin receptor desensitization

Rapid desensitization of tissues in response to activated PARs has been observed, and repeated exposure of cells to PAR agonists leads to diminished effects (Brass, 1997; Dery et al., 1998). Pretreatment of mast cells with 0.1 μM thrombin diminished the subsequent response to TRAP (50 μM), but did not affect mast cell responsiveness to compound 48/80 (Table 1). An unexpected result was obtained with the $PAR_2$-AP, SLIGRL, after desensitization of $PAR_1$ by thrombin: in contrast to TRAP, the $PAR_2$-AP-induced histamine release was augmented after prior challenge of mast cells with thrombin (Table 1).

### 3.4. PAR-mediated mast cell activation during acute peritonitis

To evaluate mast cell functions, especially the contribution of PARs, in acute inflammation, we have developed a model of thioglycolate peritonitis for rats according to the methods of Melnicoff et al. (1989) and Pejler (1999). Inflammatory peritoneal mast cells were obtained 16 h after intraperitoneal injection of thioglycolate. At this time, acute peritonitis was observed macroscopically, and the inflammatory mast cells had already lost part of their histamine and β-hexosaminidase stores. The release of β-hexosaminidase from inflammatory mast cells was significantly lower upon stimulation with compound 48/80 (5 μg/ml). To stabilize the mast cells, one group of rats was pretreated with ketotifen (1 mg/kg), which was administered intragastrically 10 h and 1 h before the thioglycolate injection. The control group received only ketotifen.

The inflammatory peritoneal mast cells from rats with and without ketotifen administration were stimulated with thrombin, TRAP, $PAR_2$-AP and compound 48/80. In spite of mast cell depletion during acute peritonitis, as shown with compound 48/80, inflammatory peritoneal mast cells displayed a higher thrombin- and TRAP-induced β-hexosaminidase release (Fig. 5). $PAR_2$-AP was also able to activate mast cells upon inflammation, but the apparent increase in the secretion of β-hexosaminidase was not statistically significant (Fig. 5).

Table 1
Histamine release from rat mast cells after preincubation with physiological saline or with thrombin (0.1 μM)

| Agonists | Percent release of histamine from mast cells after preincubation with physiological saline | Percent release of histamine from mast cells after preincubation with thrombin |
|---|---|---|
| Unstimulated release | 19.1 ± 2.3 | |
| Compound 48/80 (5 μg/ml) | 78.4 ± 5.7 | 76.6 ± 3.0 |
| TRAP (SFFLRN) (50 μM) | 41.4 ± 4.1 | 26.4 ± 5.3[a] |
| $PAR_2$-AP (SLIGRL) (10 μM) | 28.7 ± 3.1 | 42.2 ± 9.0[a] |

Data are means ± S.E.M. $n = 5$.
[a] $P < 0.05$ in comparison to secretion after preincubation without thrombin.

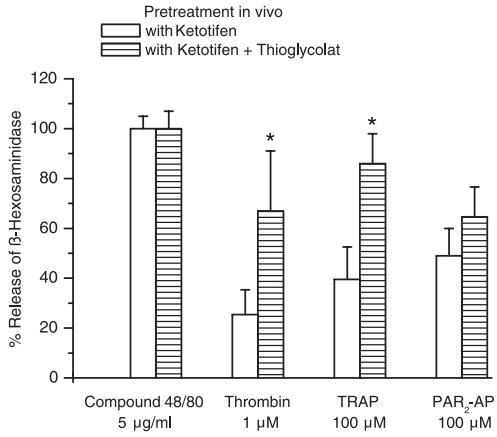


Fig. 5. Release of β-hexosaminidase from rat peritoneal mast cells during acute peritonitis. To stabilize the mast cells, the rats were pretreated with ketotifen (1 mg/kg). The histamine release induced by compound 48/80, which was significantly lower during peritonitis (from a mean value of 22%, down to 8%), was taken as 100%, and the effects of other agonists were related to amounts secretion induced by compound 48/80. Data are means ± S.E.M. from five separate experiments. *$P < 0.05$ compared to control.

## 4. Discussion

In previous studies, it was shown that thrombin causes degranulation of cultured mast cells of bone marrow and skin, and at high concentrations, it stimulates degranulation of rat peritoneal mast cells (Razin et al., 1984; Razin et al., 1985; Strukova et al., 1996; Umarova et al., 2000; Vliagoftis, 2002). Recently, it was revealed that in vitro stimulation of rat peritoneal mast cells with thrombin rapidly induced degranulation in a dose-dependent manner (Kinoshita et al., 2002). The present studies confirm the thrombin-induced mast cell activation. The $PAR_1$-AP, TRAP: SFFLRN, also induced a concentration-dependent release of mediators such as histamine from rat mast cells. Previously, it was shown that TRAP stimulates mast cells to release NO (Strukova et al., 1999). In the rat paw model of inflammation, the injection of TRAP reproduced the effects of thrombin in increasing edema and vascular permeability; degranulation of mast cells was observed histochemically (Cirino et al., 1996). It was suggested that the effects of thrombin and TRAP were mediated via activation of $PAR_1$ on mast cells. The results of our experiments with cathepsin G support this mechanism. However, in another report, thrombin and $PAR_1$-AP (SFLLR) failed to induce histamine release from peritoneal mast cells (Nishikawa et al., 2000). This discrepancy seems likely to be related to the type of thrombin and $PAR_1$-AP used or/and also to the current state of peritoneal

mast cells. It is suggested that the highly purified and stable α-thrombin from bovine plasma may induce peritoneal mast cell activation and mediator release. Instead of the selective but modified $PAR_1$-AP (as ApfFRChaCitY-$NH_2$ or TFLLR-$NH_2$), we preferred to use the unmodified form of $PAR_1$-AP, SFFLRN (murine), which is similar to the native tethered ligand of rodent $PAR_1$. Thus, we tried to demonstrate their effects on resting and inflammatory mast cells, which may express different PARs. In preliminary studies, the expression of mRNA for $PAR_1$ and $PAR_4$ was revealed in rat peritoneal mast cells (Kiseleva et al., unpublished data). Rat peritoneal mast cells also contain $PAR_2$. This was confirmed by the marked response to the $PAR_2$-AP, SLIGRL, and to the action of trypsin. Intraplantar injection of SLIGRL induces acute inflammation in part due to mast cell degranulation in the rat hindpaw (Kawabata et al., 1998). In other experiments, the acute inflammatory response mediated by $PAR_2$-APs was found to be largely independent of mast cell activation (Vergnolle et al., 1999).

Through the use of immunohistochemical techniques in human mast cells from various tissues, both $PAR_1$ and $PAR_2$ have been localized to plasma membranes and to intracellular granules (D'Andrea et al., 1999). The presence of these receptors on mast cells likely accounts for the ability of $PAR_1$-AP and $PAR_2$-AP to stimulate secretion from these cells. Pretreatment of mast cells with thrombin caused desensitization to the $PAR_1$-AP, TRAP. The response to $PAR_2$-AP was significantly increased, however, which means cross-desensitization did not occur between $PAR_1$ and $PAR_2$. It is likely that after activation of $PAR_1$ the exposure of (additional?) preformed $PAR_2$ on the mast cell surface is promoted. The results of the present experiments are the first evidence of cooperation between the two types of receptors in mast cell activation. These findings are in agreement with experiments with HEK cells which express $PAR_1$ and $PAR_2$ (Kawabata et al., 1999). It was shown that $PAR_1$ activation did not lead to heterologous desensitization of $PAR_2$ , and vice versa.

A family of polybasic molecules (compound 48/80, mastoparan and polymers of basic amino acids) is known to stimulate exocytosis from mast cells (Galli, 2000; Metcalfe et al.,1997). These polybasic secretagogues are able to release histamine by a common mechanism, and they have similar structural features that are essential for mediator-releasing activity. The most important feature is the existence of a cationic cluster at one side of the hydrophobic moiety, which may be an α-helical structure or an aromatic ring (Metcalfe et al., 1997). Our results with Mpr(Cha) can be explained by taking into account its structural characteristics: peptides such as haTRAP and Mpr(Cha) contain hydrophobic moieties and charged residues, which might be the reason for their strong degranulation activity. Consistent with findings about the stimulation of peritoneal mast cells with the $PAR_1$-AP, ApfFRChaCitY-$NH_2$ (Stenton et al., 2002), we suppose that these peptides exert their effects on mast cells not only via the PAR family of receptors. Thus, the use of native unmodified, but not so selective $PAR_1$-APs is preferred.

The possible role of PARs on mast cells during acute and chronic inflammation has recently come under intensive scrutiny (Laine et al., 1999; Pejler, 1999; Vergnolle, 2001). Mast cells isolated 16 h after acute thioglycolate-induced peritonitis have already released part of their intracellular mediators. In this study, secretion from inflammatory mast cells induced by compound 48/80 was taken as 100% because compound 48/80 at 5 μg/ml induced maximal degranulation of control and inflammatory mast cells. In comparison with the release induced by compound 48/80, the thrombin- and TRAP-induced secretion from inflammatory mast cells was similar or only slightly less than that of control, noninflammatory cells. These results suggest that $PAR_1$ expression or/and (adaptive) exposure is up-regulated in mast cells after an inflammatory stimulus in rats. An increase in $PAR_1$ expression was also found at sites of vessel injury and of atheroma rupture (Dery et al., 1998).

The thrombin-induced increase in mediator release in inflammatory mast cells (related to its secretion in response to compound 48/80) seems to be connected (i) with the additional expression of PARs on mast cells, which was manifested as an increased mast cell sensitivity to PAR agonists; (ii) with an increase in the sensitivity of the mast cells to PAR agonists, induced by changes in cytokine expression; (iii) with proteinase-mediated increase in PAR sensitivity. It was found that local cytokine production can influence the sensitivity of mast cells to non-IgE-mediated stimulation (Karimi et al., 2000). In neural cells, matrix metalloproteinase-1 (MMP-1, interstitial collagenase) can potentiate the PAR agonist-induced increase in intracellular $Ca^{2+}$ via PAR (Conant et al., 2002). In addition, a cooperation between PARs is possible, as demonstrated by the modulation of the responses of murine platelets, activated by thrombin, as possibly being the result of the complementary binding of thrombin to $PAR_3$ and followed by local proteolysis of $PAR_4$ (Nakanishi-Matsui et al., 2000).

The up-regulation of an immunologic pathway (activated by allergen upon cross-linking of FcεRI-bound IgE) by thrombin through $PAR_1$ on mast cells was shown recently (Gordon et al., 2000). The PAR-mediated activation of sensitized mast cells can stimulate responses induced by FcεRI cross-linking: at near-background levels of allergen, threshold concentrations of thrombin synergistically enhanced the release of the inflammatory cytokine interleukin-6 (IL-6) from mouse mast cells (Gordon et al., 2000). Differential expression of functional $PAR_2$ in human vascular smooth muscle cells and endothelial cells was found (Molino et al., 1998). Moreover, inflammatory stimuli such as interleukin-1 increased $PAR_2$ (but not $PAR_1$) expression only in human endothelial cells (Hamilton et al., 2001). It is of interest that disease-related

differences in receptor expression have been reported for $PAR_1$, which is expressed preferentially in endothelial and only in smooth muscle cells of atheromatous arteries (Nelken et al.,1992).

Our results speak in favor of the involvement of mast cells in inflammatory processes via specific receptors of the PAR family, namely, $PAR_1$ and $PAR_2$. Finally, the existence of adaptive regulation of PAR expression on inflammatory cells or of an increase in mast cell PAR sensitivity to PAR agonists or of the cooperation between PARs and other receptors in vivo strongly supports the hypothesis that PARs play an essential role in inflammatory processes.

## 5. Uncited reference

Vergnolle, 1999

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## References

Brass, L., 1997. Thrombin receptor antagonists: a work in progress. Coron. Artery Dis. 8, 49–58.

Camerer, E., Huang, W., Coughlin, S.R., 2000. Tissue factor- and factor X-dependent activation of protease-activated receptor 2 by factor VIIa. Proc. Natl. Acad. Sci. U. S. A. 97, 5255–5260.

Cirino, C., Cicala, C., Bucci, M.R., Sorrentino, L., Maraganore, J.M., Stone, S.R., 1996. Thrombin functions as an inflammatory mediator through activation of its receptor. J. Exp. Med. 183, 821–827.

Cocks, T.M., Moffatt, J.D., 2000. Protease-activated receptors: sentries for inflammation? Trends Pharmacol. Sci. 21, 103–108.

Conant, K., Haughey, N., Nath, A., St. Hillaire, C., Gary, D.S., Pardo, C.A., Wahl, L.M., Bilak, M., Milward, E., Mattson, M.P., 2002. Matrix metalloproteinase-1 activates a pertussis toxin-sensitive signaling pathway that stimulates the release of matrix metalloproteinase-9. J. Neurochem. 82, 885–893.

Coughlin, S.R., 1999. How the protease thrombin talks to cells. Proc. Natl. Acad. Sci. U. S. A. 96, 11023–11027.

D'Andrea, M.R., Rogahn, C.J., Andrade-Gordon, P., 2000. Localization of protease-activated receptors-1 and-2 in human mast cells: indications for an amplified mast cell degranulation cascade. Biotech. Histochem. 75, 85–90.

Dery, O., Corvery, C.U., Steinhorff, M., Bunnett, N.W., 1998. Proteinase-activated receptor: novel mechanisms of signaling by serin proteases. Am. J. Physiol. 274, 1429–1452.

Feng, D.-M., Veber, D.F., Connolly, T.M., Condra, C., Tang, M.-J., Nutt, R.F., 1995. Development of a potent thrombin receptor ligand. J. Med. Chem. 38, 4125–4130.

Galli, S.J., 2000. Mast cells and basophils. Curr. Opin. Hematol. 7, 32–39.

Gordon, J.R., Zhang, X., Stevenson, K., Cosford, K., 2000. Thrombin induces IL-6 but not TNFa secretion by mouse mast cells: threshold-level thrombin receptor and very low level FcγRI signaling synergistically enhance IL-6 secretion. Cell. Immunol. 205, 128–135.

Hamilton, J.R., Frauman, A.G., Cocks, T.M., 2001. Increased expression of proteinase-activated receptor-2 (PAR2) and PAR4 in human coronary artery by inflammatory stimuli unveils endothelium-dependent relaxations to PAR2 and PAR4 agonists. Circ. Res. 89, 92–98.

Karimi, K., Redegeld, F.A., Blom, R., Nijkamp, F.P., 2000. Stem cell factor and interleukin-4 increase responsiveness of mast cells to substance P. Exp. Hematol. 28, 626–634.

Kawabata, A., Kuroda, R., Minami, T., Kataoka, K., Taneda, M., 1998. Increased vascular permeability by a specific agonist of proteinase-activated receptor-2 in rat hindpaw. Br. J. Pharmacol. 125, 419–422.

Kawabata, A., Saifedine, M., Al-Ani, B., Leblond, L., Hollenberg, M.D., 1999. Evaluation of proteinase-activated receptor-1 (PAR-1) agonists and antagonists using a cultured cell receptor desensitization assay: activation of PAR-2 by PAR-1-targeted ligands. J. Pharmacol. Exp. Ther. 288, 358–370.

Kinoshita, M., Miyamoto, T., Ohashi, N., Sasayama, S., Matsumori, A., 2002. Thrombosis increases circulatory hepatocyte growth factor by degranulation of mast cells. Circulation 106, 3133–3138.

Laine, P., Kaartinen, M., Penttila, A., Panula, P., Paavonen, T., Kovanen, P.T., 1999. Association between myocardial infarction and the mast cells in the adventitia of the infarct-related coronary artery. Circulation 99, 361–369.

Melnicoff, M.J., Horon, P.K., Morahan, P.S., 1989. Kinetics of changes in peritoneal cell populations following acute inflammation. Cell. Immunol. 118, 178–191.

Metcalfe, D.D., Baram, D., Mekori, Y.A., 1997. Mast cells. Physiol. Rev. 77, 1033–1075.

Molino, M., Blanchard, N., Belmonte, E., Tarver, A.P., Abrams, C., Hoxie, J.A., Cerletti, C., Brass, L.F., 1995. Proteolysis of the human platelet and endothelial cell thrombin receptor by neutrophil-derived cathepsin G. J. Biol. Chem. 270, 11168–11175.

Molino, M., Raghunath, P.N., Kuo, A., Ahuja, M., Hoxie, J.A., Brass, L.F., Barnathan, E.S., 1998. Differential expression of functional protease-activated receptor-2 (PAR-2) in human vascular smooth muscle cells. Arterioscler. Thromb. Vasc. Biol. 18, 825–832.

Nakanishi-Matsui, M., Zheng, Y.W., Sulciner, D.J., Weiss, E.J., Ludeman, M.J., Coughlin, S.R., 2000. PAR3 is a cofactor for PAR4 activation by thrombin. Nature 404, 609–613.

Nelken, N., Soifer, S., O'Keefe, J., Vu, T.-K., Charo, I., Coughlin, S., 1992. Thrombin receptor expression in normal and atherosclerotic human arteries. J. Clin. Invest. 90, 1614–1621.

Nishikawa, H., Kawabata, A., Kuroda, R., Nishida, M., Kawai, K., 2000. Characterization of protease-activated receptors in rat peritoneal mast cells. Jpn. J. Pharmacol. 82, 74–77.

Pejler, G., 1999. Procoagulant and anticoagulant activities of resident and inflammatory peritoneal cells. Inflamm. Res. 48, 344–350.

Razin, E., Marx, G., 1984. Thrombin-induced degranulation of cultured bone marrow-derived mast cells. J. Immunol. 133, 3282–3285.

Razin, E., Baranes, D., Marx, G., 1985. Thrombin-mast cell interactions. Binding and cell activation. Exp. Cell Res. 160, 380–386.

Schwartz, L.B., Austen, K.F., Wasserman, S.I., 1982. Immunologic release of β-hexosaminidase and β-glucuronidase from purified rat serosal mast cells. J. Immunol. 123, 1445–1450.

Shore, P.A., 1971. The chemical determination of histamine. Methods Biochem. Anal., 89–97.

Stenton, G.R., Nohara, O., D'ery, R.E., Vliagoftis, H., Gilchrist, M., Johri, A., Wallace, J.L., Hollenberg, M.D., Moqbel, R., Befus, A.D., 2002. Proteinase-activated receptor (PAR)-1 and-2 agonists induce mediator release from mast cells by pathways distinct from PAR-1 and PAR-2. J. Pharmacol. Exp. Ther. 302, 466–474.

Strukova, S.M., 2001. Thrombin as a regulator of inflammation and reparative processes in tissues. Biochemistry (Moscow) 66, 8–18.

Strukova, S.M., Dugina, T.N., Khlgatian, S.V., Redkozubov, A.E., Redkozubova, G.P., Pinelis, V.G., 1996. Thrombin-mediated events implicated in mast cell activation. Semin. Thromb. Hemost. 22, 145–150.

Strukova, S., Chistov, I., Umarova, B., Dugina, T., Storojevikh, T., Pinelis, V., Glusa, E., 1999. Modulation of mast cell activity by a petide agonist of the thrombin receptor: role of nitric oxide. Biochemistry (Moscow) 64, 658–664.

Umarova, B.A., Dugina, T.N., Shestakova, E.V., Glusa, E., Strukova, S.M., 2000. Activation of rat mast cells upon stimulation of protease-activated receptor (PAR-1). Bull. Exp. Biol. Med. 129, 314–317.

Vergnolle, N., 1999. Proteinase-activated receptor-2-activating peptides induce leukocyte rolling, adhesion, and extravasation in vivo. J. Immunol. 163, 5064–5069.

Vergnolle, N., Hollenberg, M., Sharkey, K.A., Wallace, J., 1999. Characterization of the inflammatory response to proteinase-activated receptor-2 (PAR2)-activating peptides in the rat paw. Br. J. Pharmacol. 127, 1083–1090.

Vergnolle, N., Wallace, J., Bunnett, N., Hollenberg, M., 2001. Protease-activated receptors in inflammation, neuronal signaling and pain. Trends Pharmacol. Sci. 22, 146–152.

Vliagoftis, H., 2002. Thrombin induces mast cell adhesion to fibronectin: evidence for involvement of protease-activated receptor-1. J. Immunol. 169, 4551–4558.