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Enhancement of vascular permeability by specific activation of protease-activated receptor-1 in rat hindpaw: a protective role of endogenous and exogenous nitric oxide

*,[1]Atsufumi Kawabata, [1]Ryotaro Kuroda, [2]Hiroyuki Nishikawa, [3]Toshiharu Asai, [3]Kazuo Kataoka & [3]Mamoru Taneda

[1]Department of Pathophysiology and Therapeutics, Faculty of Pharmaceutical Sciences, Kinki University, 3-4-1 Kowakae, Higashi-Osaka 577-8502, Japan; [2]Research and Development Center, Fuso Pharmaceutical Industries Ltd., Osaka 536-0025, Japan and [3]Department of Neurosurgery, Faculty of Medicine, Kinki University, Osaka-Sayama 589-8511, Japan

1 To clarify the role of the first thrombin receptor/protease-activated receptor (PAR)-1 in an inflammatory process, we tested and characterized the effect of intraplantar (i.pl.) administration of the highly specific PAR-1 agonist TFLLR-NH_2 in rat hindpaw.

2 TFLLR-NH_2 administered i.pl. at 0.01–0.03 μmol per paw enhanced vascular permeability in the hindpaw and produced paw oedema in a dose-dependent manner. This effect was almost completely abolished by repeated pretreatment with compound 48/80 to deplete inflammatory mediators in mast cells.

3 The NO synthase inhibitor N^G-nitro-L-arginine methyl ester or N-iminoethyl-L-ornithine, preadministered i.pl., stereospecifically potentiated the i.pl. TFLLR-NH_2-induced permeability increase, while the NO donor sodium nitroprusside or NOC-18, given i.pl., suppressed the effect of TFLLR-NH_2.

4 These findings demonstrate that specific activation of PAR-1 produces increased vascular permeability accompanied by oedema formation in the rat hindpaw, predominantly *via* mast cell degranulation, and that endogenous and exogenous NO plays a protective role in the PAR-1-mediated inflammatory event.

Keywords: Protease-activated receptor (PAR); protease; thrombin; nitric oxide; nitric oxide synthase; vascular permeability; inflammation; oedema; mast cell degranulation

Abbreviations: D-NAME, N^G-nitro-D-arginine methyl ester; 5-HT, 5-hydroxytryptamine; i.pl., intraplantar; L-NAME, N^G-nitro-L-arginine methyl ester; L-NIO, N-iminoethyl-L-ornithine; NO, nitric oxide; NP, sodium nitroprusside; P14, SFLLRNPNDKYEPF; PAR, protease-activated receptor

Introduction

Thrombin receptors belong to a new family of G-protein coupled, seven trans-membrane domain receptors, the activation of which occurs following proteolytic cleavage of the extracellular N-terminus of the receptor and subsequent interaction of the exposed 'tethered ligand' with some other region of the receptor (Vu *et al*., 1991; Nystedt *et al*., 1994; Bohm *et al*., 1996; Ishihara *et al*., 1997; Hollenberg, 1996). This family of protease-activated receptors (PAR) mediates the cellular actions of various proteases including thrombin and trypsin. PAR-1 is the first thrombin receptor, and the active sequence of the N-terminus that appears after the proteolytic cleavage is SFLLRNPNDKYEPF – for human PAR-1 (Vu *et al*., 1991; Hollenberg, 1996). Interestingly, exogenously applied synthetic peptides as short as 5 amino acids based on the N-terminus sequence of the tethered ligand of PAR-1, such as SFLLR, can fully activate PAR-1 and mimic many of cellular actions of thrombin (Muramatsu *et al*., 1992; Blackhart *et al*., 1996; Hollenberg *et al*., 1996; 1997; Kawabata *et al*., 1997; 1999). PAR-2, the second member of this family of receptors, does not respond to thrombin but reacts to trypsin (Nystedt *et al*., 1994; Kawabata *et al*., 1999). Some known or unknown enzymes distinct from trypsin have also been considered as the endogenous agonist enzyme of PAR-2, because PAR-2 is distributed even in tissues not normally exposed to endogenous trypsin (Bohm *et al*., 1996; Fox *et al*., 1997; Molino *et al*., 1997). The physiological and/or pathophysiological roles of PAR-2 are not yet well understood (Nystedt *et al*., 1994; Bohm *et al*., 1996; Hollenberg *et al*., 1996). PAR-3 and PAR-4 have been cloned as the second and third thrombin receptor, respectively, in both humans and rodents (Ishihara *et al*., 1997; Kahn *et al*., 1998; Xu *et al*., 1998). However, the detailed activation mechanisms and/or physiological roles of these receptors remain to be investigated.

PAR-1 is involved in a variety of biological events including human platelet aggregation, vascular contraction/relaxation (Muramatsu *et al*., 1992; Laniyonu & Hollenberg, 1995) and an increase in endothelial permeability (Malik & Fento, 1992). An *in vivo* study (Cirino *et al*., 1996) has demonstrated that intraplantar (i.pl.) administration of thrombin or SFLLRNPNDKYEPF (P14), a PAR-1-activating peptide based on the receptor-activating sequence of human PAR-1, produces mast cell degranulation resulting in increased vascular permeability and oedema formation in rat hindpaw, while SFLLANPNDKYEPF, an inactive control peptide, does not. This finding is in agreement with the *in vitro* evidence that thrombin induces degranulation of cultured bone marrow-derived mast cells (Razin & Marx, 1984). PAR-1 thus appears to play a role in the inflammatory process. However, P14 has poor specificity for PAR-1, because it potently activates not only PAR-1 but also PAR-2 (Kawabata *et al*., 1999). Most recently we have found that

*Author for correspondence.

SLIGRL-NH_2, an entirely specific agonist of PAR-2, when administered i.pl., mimics the actions of i.pl. thrombin or P14 in the rat hindpaw (Kawabata *et al*., 1998). Therefore, the possibility cannot be excluded that PAR-2 is involved in P14-induced inflammation. In addition, thrombin also exerts a number of biological actions independent of PAR-1 including the activation of PAR-3 and PAR-4 (Ishihara *et al*., 1997; Kahn *et al*., 1998; Xu *et al*., 1998). To test for an essential role of PAR-1 in inflammation, therefore, it is necessary to examine the effect of specific PAR-1 agonists in the rat hindpaw system. In the present study, we thus evaluated the effects of TFLLR-NH_2, a highly specific and potent PAR-1 agonist synthesized on the basis of the active sequences of the human and *Xenopus* PAR-1 (Blackhart *et al*., 1996; Hollenberg *et al*., 1997; Kawabata *et al*., 1997; 1999), on vascular permeability in the rat hindpaw, and then examined an involvement of mast cell degranulation in the PAR-1-mediated inflammatory responses.

Nitric oxide (NO) plays an important but complex role in the inflammatory process (Miller *et al*., 1993; Kawabata *et al*., 1994). Activation of the NO-cyclic GMP pathway *in vitro* decreases the permeability of cultured endothelial monolayers possibly *via* an increase in cell-cell contact by relaxing the endothelial cytoskeleton, but not in all circumstances (for review, see Warren, 1993). On the other hand, *in vivo*, NO appears to be involved, at least partially, in the increased permeability or oedema formation caused by carrageenin, bradykinin, substance P and 5-hydroxytryptamine, while it may play a minor role in the inflammatory responses to histamine (Hughes *et al*., 1990; Ialenti *et al*., 1992; Teixeira *et al*., 1993; Paul *et al*., 1994; Giraldelo *et al*., 1994; Fujii *et al*., 1994; 1995). There is also *in vivo* evidence that a NO synthase inhibitor enhances microvascular permeability (Kubes & Granger, 1992). In the inflammatory models induced by lipopolysaccharide or platelet-activating factor, NO plays a dual role, being pro-inflammatory or protective depending on the experimental conditions including differences in the species or tissues employed (Filep & Foldes-Filep, 1993; Filep *et al*., 1993; Boughton-Smith *et al*., 1993; Laszlo *et al*., 1995; Fujii *et al*., 1995). Thus, the relationship between NO and inflammation is very complex and still remains to be interpreted. The present study also investigated the role of NO in the increased vascular permeability induced by specific activation of PAR-1 in the rat hindpaw. Here we describe that endogenous and exogenous NO is protective against the PAR-1-mediated increase in vascular permeability.

Methods

Experimental animals

Male Wistar rats weighing 150–250 g (Japan SLC. Inc., Japan) were used throughout the present experiments. They were maintained on a 12 h light-dark cycle on a standard laboratory diet and tap water *ad libitum* before experiments.

Determination of vascular permeability and oedema formation in the rat hindpaw, and intraplantar (i.pl.) administration of TFLLR-NH_2, a specific PAR-1 agonist

Under anaesthesia by intraperitoneal (i.p.) urethane at 1.5 g kg^{-1}, vascular permeability was determined as described previously (Cirino *et al*., 1996; Kawabata *et al*., 1998). The rats received an intravenous (i.v.) injection of 2.5% w v^{-1} Evans blue solution in 0.45% NaCl at a dose of 25 mg kg^{-1}, immediately before intraplantar (i.pl.) injection of the specific PAR-1 agonist TFLLR-NH_2 at 0.01–0.1 μmol per paw, or its vehicle (physiological saline) in a volume of 100 μl into the left hindpaw. After 15 min, the rats were killed by decapitation, and both the drug-treated and contralateral hindpaws were removed and minced. Each paw was incubated in 10 ml of formamide for 72 h at 37°C to extract the dye, and thereafter the solution was filtered. The content of Evans blue in each hindpaw was quantified by measuring the optical density of the filtrate at 619 nm. The amount of Evans blue extravasated was defined as the difference between the contents in the left (drug-treated) and right (untreated) hindpaws, based upon the preliminary data that i.pl. administration of TFLLR-NH_2 at 0.1 μmol per paw did not modify vascular permeability in the contralateral hindpaw. In some experiments, the size of oedema was determined by measuring the thickness of the hindpaw immediately before and 15 min after the i.pl. injection of TFLLR-NH_2, using a tissue caliper with 0.05 mm accuracy (Herzberg *et al*., 1994). Oedema formation expressed as percentage change of the value measured before the i.pl. injection. The time schedules used in the present study were based on preliminary data that the effect of TFLLR-NH_2 at 0.03 μmol per paw peaked 15 min after i.pl. administration, in agreement with a previous report employing P14 (Cirino *et al*., 1996).

Depletion of bioactive amines in mast cells

Depletion of mast cell histamine and 5-hydroxytryptamine was achieved by chronic treatment of the rats with the mast cell degranulator compound 48/80 as described previously (Di Rosa *et al*., 1971; Kawabata *et al*., 1998). Briefly, the rats received i.p. compound 48/80 (0.1% w v^{-1} in saline) in the morning and evening for eight doses, starting with an evening dose. The dose used was 0.6 mg kg^{-1} for the first six administrations and 1.2 mg kg^{-1} for the last two administrations. Control rats received i.p. vehicle (saline only) according to the same schedule. Vascular permeability in the depleted animals was assayed as described above, except that the contralateral hindpaw was injected i.pl. with saline in the same volume. Specific extravasation of Evans blue was determined by subtracting the Evans blue content in the saline-treated contralateral hindpaw from that in the drug-treated hindpaw, since the chronic treatment with compound 48/80 slightly affected the magnitude of the i.pl. saline-induced small increase in vascular permeability; the Evans blue content (μg per paw) in the non-injected paw was 9.79 ± 0.45 and 9.83 ± 0.32 in rats repeatedly treated with vehicle and compound 48/80, respectively, and that in the i.pl. saline-injected paw was 22.67 ± 2.19 and 14.07 ± 3.37 in rats treated with vehicle and compound 48/80, respectively ($n=4$). In preliminary experiments, the complete depletion of mast cell amines by these procedures was checked by confirming the absence of oedema for 1 h after i.pl. carrageenin at 1 mg per paw ($n=4$), and it was also checked that the treatment failed to modify the increased permeability due to i.pl. histamine at 100 nmol per paw, in agreement with our previous results (Kawabata *et al*., 1998).

Evaluation of the effects of various drugs preadministered i.pl. on the i.pl. TFLLR-NH_2-induced increase in vascular permeability of the rat hindpaw

N^G-nitro-L-arginine methyl ester (L-NAME) at 0.1–1 μmol per paw, N-iminoethyl-L-ornithine (L-NIO) at 1 μmol per paw

or N^G-nitro-D-arginine methyl ester (D-NAME) at 1 μmol per paw, in a volume of 20 μl, was administered i.pl. 30 s before the i.v. injection of Evans blue. Sodium nitroprusside at 0.1–1 μmol per paw, NOC-18 at 1–2 μmol per paw, L-arginine or D-arginine, at 1–10 μmol per paw, was also given i.pl. in the same manner. Control animals received i.pl. vehicle (saline) in the same volume and administration schedule. The administration schedules employed here followed those used in related reports (Hughes *et al*., 1990; Ialenti *et al*., 1992; Teixeira *et al*., 1993; Giraldelo *et al*., 1994).

Chemicals employed

All drugs, TFLLR-NH_2 (Peptide Synthesis Core Facility, Department of Medical Biochemistry, University of Calgary, Canada; M.W. 647.8), compound 48/80, N^G-nitro-L-arginine methyl ester hydrochloride, N^G-nitro-D-arginine methyl ester hydrochloride, L-arginine hydrochloride, D-arginine hydrochloride (Sigma, U.S.A.), N-iminoethyl-L-ornithine hydrochloride (Peptide Institute, Japan), sodium nitroprusside (Kishida Chemicals, Japan), and NOC-18 [2,2′-(hydroxynitrosohydrazine)*bis*-ethanamine; Calbiochem, U.S.A.] were dissolved in physiological saline.

Statistical analyses

All data obtained are expressed as means ± s.e.mean. Statistical analyses were performed by Student's unpaired *t*-test for comparison between two-group data or by Newman–Keuls' test for multiple-group comparison. The difference between groups was considered significant when $P<0.05$.

Results

Enhanced vascular permeability and oedema formation caused by i.pl. administration of the specific PAR-1 agonist TFLLR-NH_2 in the rat hindpaw

TFLLR-NH_2, a specific PAR-1 agonist, when administered i.pl. at 0.01, 0.03 and 0.1 μmol per paw, produced a rapid increase in vascular permeability in the rat hindpaw as assessed by the Evans blue extravasation technique, the effect being dose-dependent (Figure 1, left panel). The i.pl. administration of TFLLR-NH_2 in the same dose range also led to oedema formation in the hindpaw in a dose-dependent manner (Figure 1, right panel).

Lack of effect of i.pl. TFLLR-NH_2 on hindpaw vascular permeability in rats previously depleted of their stores of histamine and 5-hydroxytryptamine

In the rats pretreated repeatedly with compound 48/80, the TFLLR-NH_2 (0.03 μmol per paw, i.pl.)-evoked specific extravasation of Evans blue in the hindpaw was dramatically reduced. The magnitude of the effect of TFLLR-NH_2 in the depleted rats was approximately one-fifth of that in the control rats (Figure 2), indicating a primary role of mast cell

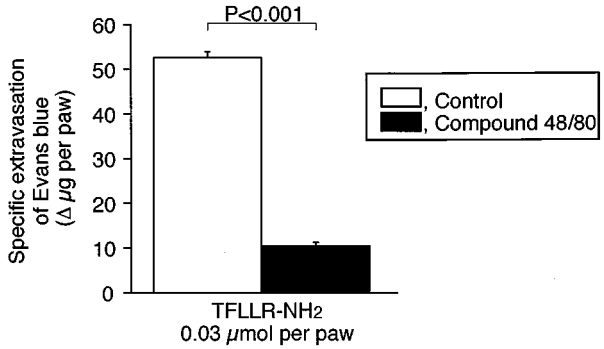

Figure 2 Lack of effect of intraplantar TFLLR-NH_2 on vascular permeability in the hindpaw of rats pretreated repeatedly with compound 48/80. Rats were repeatedly treated with compound 48/80 to deplete bioactive amines in mast cells, and thereafter received i.pl. TFLLR-NH_2 at 0.03 μmol per paw in the left hindpaw and i.pl. saline in the contralateral hindpaw, immediately after intravenous Evans blue at 25 mg kg^{-1}, and were killed after 15 min. Specific extravasation of Evans blue was defined as the difference between the Evans blue content in the drug-treated paw and that in the saline-treated contralateral paw. Data show the mean ± s.e.mean from four rats.

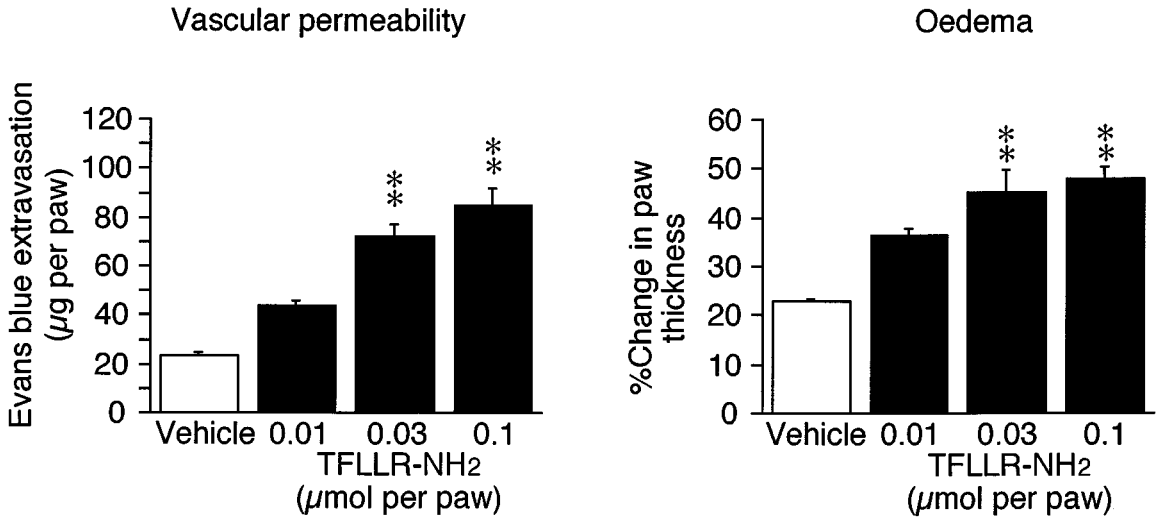

Figure 1 Increased vascular permeability and oedema caused by intraplantar TFLLR-NH_2 in the rat hindpaw. The rats received an i.pl. injection of TFLLR-NH_2 at 0.01, 0.03 or 0.1 μmol per paw immediately after intravenous Evans blue at 25 mg kg^{-1}, and were killed after 15 min. Evans blue extravasation was determined by subtracting the Evans blue content in the untreated contralateral hindpaw from that in the drug-treated hindpaw. Paw thickness was measured immediately before and 15 min after TFLLR-NH_2. Data show the mean with s.e.mean from 4–6 rats. $^{**}P<0.01$ vs the vehicle-treated rats.

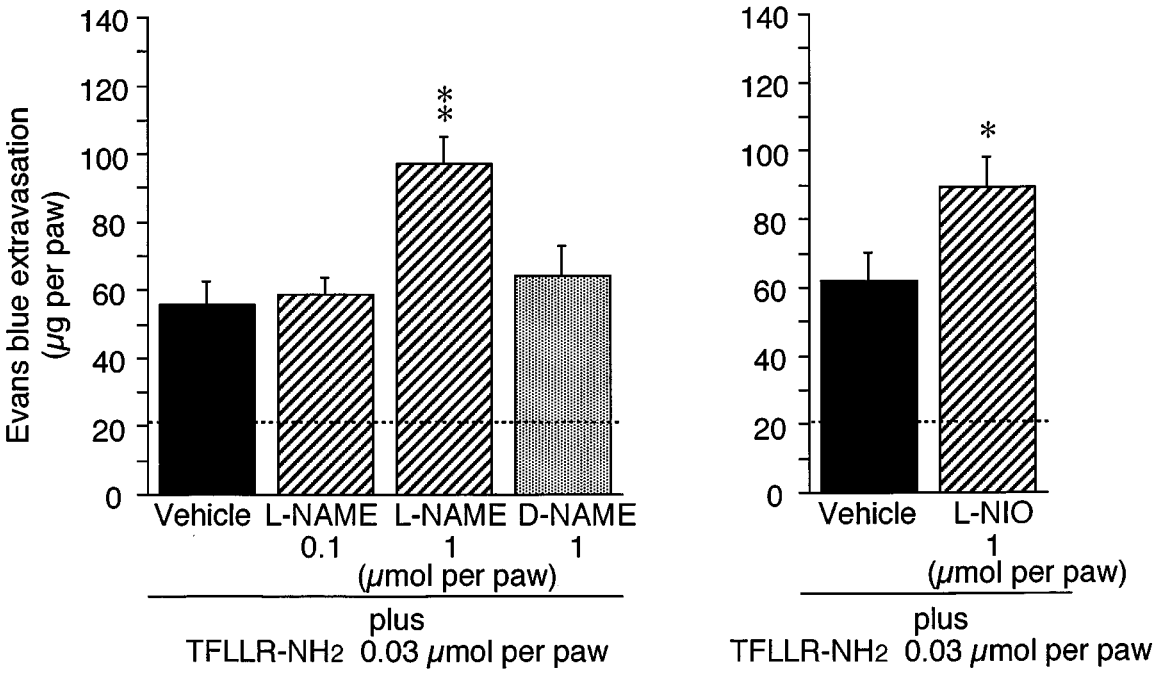

Figure 3 Enhancement of the effect of intraplantar TFLLR-NH_2 by intraplantar NO synthase inhibitors in the rat hindpaw. N^G-nitro-L-arginine methyl ester (L-NAME) at 0.1–1 μmol per paw, its D-enantiomer (D-NAME) at 1 μmol per paw or N-iminoethyl-L-ornithine at 1 μmol per paw was administered i.pl. 30 s before the i.v. injection of Evans blue at 25 mg kg^{-1}. Then the rats received an i.pl. injection of TFLLR-NH_2 at 0.03 μmol per paw immediately after i.v. Evans blue, and were killed after 15 min. Evans blue extravasation was determined by subtracting the Evans blue content in the untreated contralateral hindpaw from that in the drug-treated hindpaw. Broken lines indicate the level of Evans blue extravasation caused by saline administered i.pl. in a volume of 100 μl instead of TFLLR-NH_2. Data show the mean with s.e.mean from 4–6 rats. $^*P<0.05$, $^{**}P<0.01$ vs the group treated with vehicle plus TFLLR-NH_2.

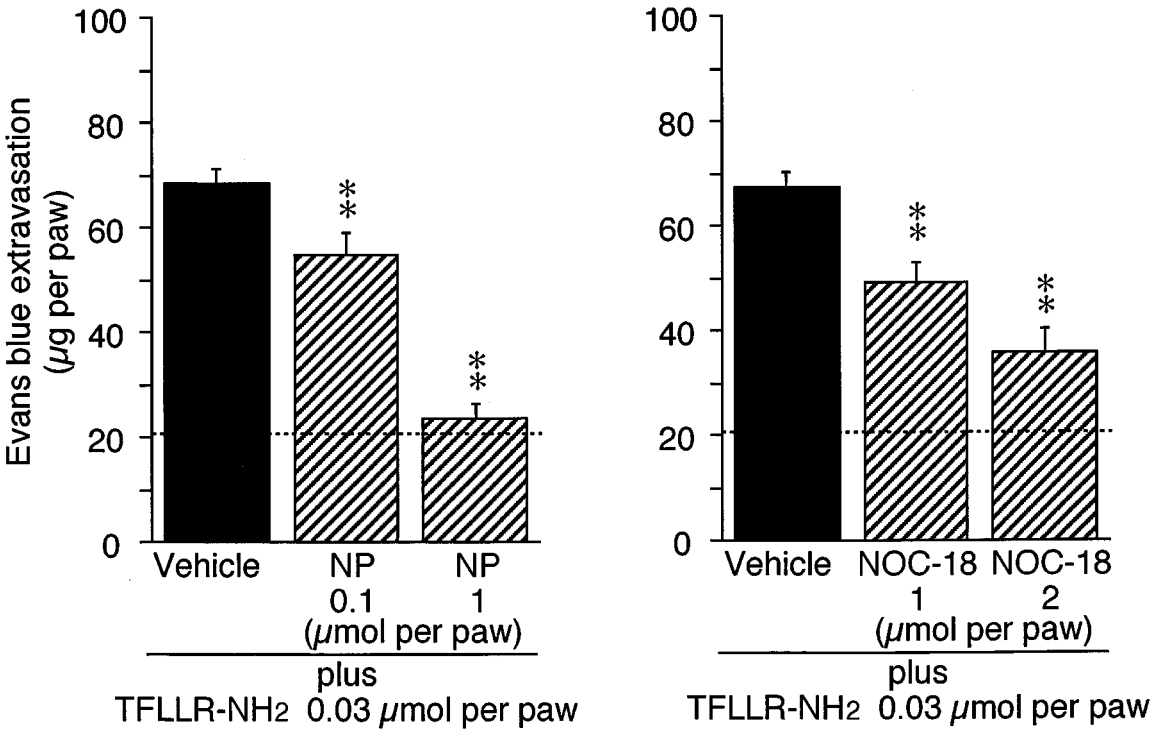

Figure 4 Suppression of the effect of intraplantar TFLLR-NH_2 by intraplantar NO donors in the rat hindpaw. Sodium nitroprusside (NP) at 0.1–1 μmol per paw or NOC-18 at 1–2 μmol per paw was administered i.pl. 30 s before the i.v. injection of Evans blue at 25 mg kg^{-1}. Then the rats received an i.pl. injection of TFLLR-NH_2 at 0.03 μmol per paw immediately after i.v. Evans blue, and were killed after 15 min. Evans blue extravasation was determined by subtracting the Evans blue content in the untreated contralateral hindpaw from that in the drug-treated hindpaw. Broken lines indicate the level of Evans blue extravasation caused by saline administered i.pl. in a volume of 100 μl instead of TFLLR-NH_2. Data show the mean ± s.e.mean from 4–6 rats. $^{**}P<0.01$ vs the group treated with vehicle plus TFLLR-NH_2.

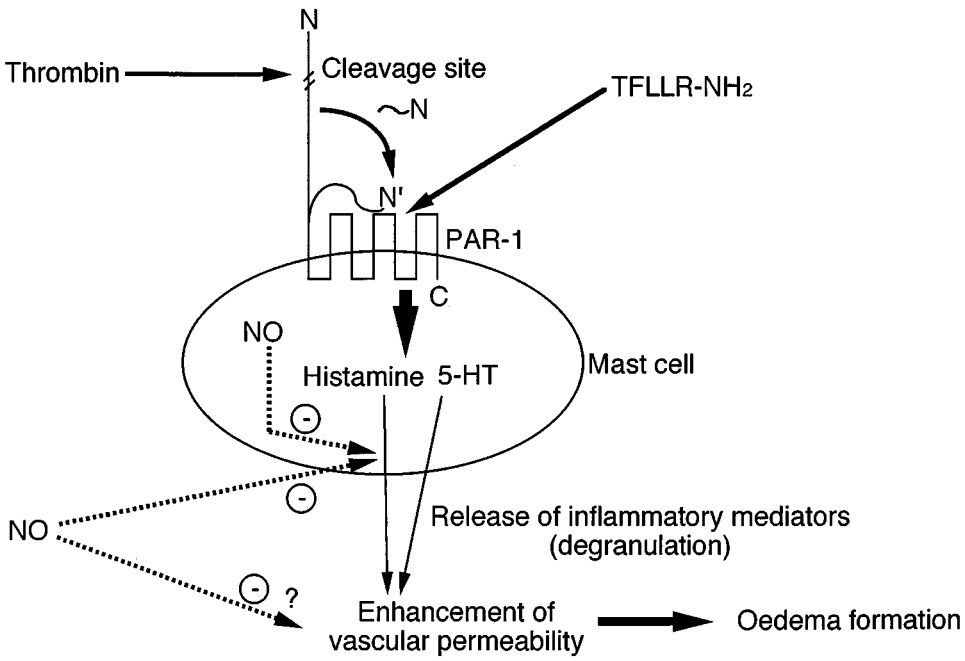

Figure 5 A proposed mechanism of PAR-1 mediated inflammation and its modulation by NO. 5-HT, 5-hydroxytryptamine. A minus sign indicates 'inhibition'.

degranulation in the PAR-1-mediated inflammatory mechanisms, supporting the study of Cirino *et al.* (1996) where agonist peptides with poor specificity for PAR-1 were only available.

Facilitation by NO synthase inhibitors, preadministered i.pl., of the i.pl. TFLLR-NH_2-induced increase in vascular permeability in the rat hindpaw

N^G-nitro-L-arginine methyl ester (L-NAME), a NO synthase inhibitor, when preadministered i.pl. at 1 μmol per paw, significantly and considerably enhanced the increase in Evans blue extravasation in the hindpaw elicited by subsequent i.pl. administration of TFLLR-NH_2 at 0.03 μmol per paw, although L-NAME at a dose of 0.1 μmol per paw had no such an effect. In contrast, its D-enantiomer (D-NAME), administered i.pl. at 1 μmol per paw, failed to alter the i.pl. TFLLR-NH_2-induced increase in vascular permeability (Figure 3, left panel). Likewise, N-iminoethyl-L-ornithine (L-NIO), another NO synthase inhibitor, when given at 1 μmol per paw in the same fashion, significantly augmented the effect of i.pl. TFLLR-NH_2 in the rat hindpaw (Figure 3, right panel).

Suppression by NO donors, preadministered i.pl., of the i.pl. TFLLR-NH_2-induced increase in vascular permeability in the rat hindpaw

The NO donor sodium nitroprusside (NP) at 0.1 and 1 μmol per paw, when administered i.pl. prior to i.pl. TFLLR-NH_2 at 0.03 μmol per paw, significantly reduced the TFLLR-NH_2-evoked enhancement of the hindpaw vascular permeability in a dose-dependent manner. In particular, the effect exerted by TFLLR-NH_2 almost completely disappeared by pretreatment with 1 μmol per paw of NP (Figure 4, left panel). NOC-18, another NO donor that is of a relatively long-acting type, preadministered i.pl. at 1–2 μmol per paw, also significantly attenuated the effect of TFLLR-NH_2 on vascular permeability in a dose-dependent manner (Figure 4, right panel).

Enhancement by i.pl. pretreatment with large doses of L- and D-arginine of the effect of i.pl. TFLLR-NH_2 on hindpaw vascular permeability

I.pl. pre-administration of L-arginine, an endogenous source of NO, at 1 μmol per paw, had no effect on the i.pl. TFLLR-NH_2-elicited increase of vascular permeability in the rat hindpaw. A larger dose, 10 μmol per paw, of L-arginine significantly potentiated the effect of TFLLR-NH_2 on hindpaw permeability. The same dose (10 μmol per paw) of i.pl. D-arginine, however, also significantly augmented the increase in vascular permeability evoked by i.pl. TFLLR-NH_2 ($n=4$, data not shown). Considering these non-specific effects of arginine itself in the present experimental conditions, we did not perform experiments confirming the reversal by L-arginine at large doses of the effects of NO synthase inhibitors.

Discussion

The present study demonstrates that specific activation of PAR-1 enhances vascular permeability and produces oedema almost exclusively through mast cell degranulation in the hindpaw of rats, indicating a close relationship between PAR-1 and inflammation. The findings that NO synthase inhibitors enhanced, whereas NO donors reduced, the PAR-1 agonist TFLLR-NH_2-induced increase in vascular permeability in the rat hindpaw, suggest that both endogenous and exogenous NO exerts a protective effect against the PAR-1-mediated inflammatory events.

Cirino *et al.* (1996) have reported that the PAR-1/thrombin receptor agonist SFLLRNPNDKYEPF (P14), but not the inactive control peptide SFLLANPNDKYEPF, dose-dependently induced oedema in the rat hindpaw by i.pl. administration at doses of 10, 100, and 500 μg (approximately 5.8, 58, 288 nmol, respectively) per paw, the effect peaking after 15 min and disappearing after 60 min. However, the selectivity of P14-NH_2 for PAR-1 relative to PAR-2 is only four as

evaluated by a cultured cell receptor desensitization assay (Kawabata *et al*., 1999), suggesting that the effect of P14 at high concentrations in the rat hindpaw assay is not necessarily attributable to activation of PAR-1 only, because our most recent study found that oedema formation and increase in vascular permeability also takes place after i.pl. administration of the exclusively specific PAR-2 agonist SLIGRL-NH_2 at 10–100 nmol per paw (Kawabata *et al*., 1998). In the present study, the highly specific PAR-1 agonist TFLLR-NH_2, administered i.pl., dose-dependently produced a rapid and transient increase in vascular permeability in a dose range of 0.01–0.1 μmol (10–100 nmol) per paw. The selectivity of TFLLR-NH_2 for PAR-1 relative to PAR-2 is 220 (Kawabata *et al*., 1999), implying that the effect of i.pl. TFLLR-NH_2 on the hindpaw permeability is almost exclusively attributable to activation of PAR-1 under the present experimental conditions. Specific antagonists of PAR-1 and PAR-2 are still not generally available at present, although some candidates have been reported (Bernatowicz *et al*., 1996). The development of antagonists with satisfactory characteristics would be beneficial to further confirm the role of PAR-1 as well as PAR-2 in inflammation.

Thrombin as well as PAR-1 activating peptides such as P14 increase permeability in monolayers of human umbilical vein endothelial cells (Schaeffer *et al*., 1997). However, this direct mechanism does not appear to contribute to the *in vivo* increased vascular permeability caused by TFLLR-NH_2 in the plantar region of the rat hindpaw in the present study. The finding that the i.pl. TFLLR-NH_2-induced increase in vascular permeability was almost completely abolished by repeated pretreatment with compound 48/80 to deplete mast cell inflammatory amines implies that the effect of i.pl. TFLLR-NH_2 predominantly results from mast cell degranulation. Thrombin or PAR-1-activating peptides when administered intravenously are likely to exert a direct action on the vascular endothelium resulting in enhancement of *in vivo* vascular permeability, but will also lead to circulatory disturbance due to intravascular coagulation and/or platelet aggregation in most species. It may also be possible that the PAR-1-activating peptides exert their effect by acting on a receptor-independent site on the surface of the mast cell membrane that is sensitive to compound 48/80. However, the involvement of PAR-1 in mast cell activation is strongly supported by our latest experiments that detected mRNA for PAR-1 in rat peritoneal mast cells by the reverse-transcriptase polymerase chain reaction (unpublished data).

It was surprising that NO synthase inhibitors, L-NAME and L-NIO, preadministered i.pl., stereospecifically augmented the i.pl. TFLLR-NH_2-induced permeability increase in the rat hindpaw, since there is a number of *in vivo* studies demonstrating suppression by NO synthase inhibitors of the increased permeability and/or oedema formation in response to carrageenin, bradykinin, substance P or 5-hydroxytryptamine (Hughes *et al*., 1990; Ialenti *et al*., 1992; Teixeira *et al*., 1993; Paul *et al*., 1994; Giraldelo *et al*., 1994; Fujii *et al*., 1994; 1995). Endogenous NO thus tonically exerts a protective effect in the PAR-1-mediated inflammatory process, suggesting a complex relationship between NO and inflammation that may be distinct according to inflammatory stimulators or other experimental conditions. Consistently, the findings that NO donors, sodium nitroprusside and NOC-18, preadministered i.pl., considerably reduced the i.pl. TFLLR-NH_2-induced increase in hindpaw permeability imply that exogenously applied NO also suppresses the PAR-1-mediated inflammation. By contrast, i.pl. preadministration of L-arginine, an endogenous source of NO, at a large dose, 10 μmol per paw, but not at 1 μmol per paw, significantly potentiated the i.pl. TFLLR-NH_2-induced permeability increase in the hindpaw. This effect of L-arginine, however, does not appear to involve NO, but is possibly due to non-specific actions of the compound, because the enantiomer D-arginine at the same large dose also exhibited a similar potentiating effect. Actually, it has been reported that arginine analogues including L-arginine, D-arginine, L-NAME and D-NAME, when administered i.pl. in a large dose, 15 μmol per paw, non-specifically facilitate the formation of paw oedema induced by bradykinin or 5-hydroxytryptamine and that the facilitation observed is probably due to the cationic charge of these substances (Giraldelo *et al*., 1994). The mechanisms underlying the inhibitory actions of endogenous and exogenous NO on the PAR-1 mediated permeability increase remain to be investigated. The most likely mechanism is suppression by NO of the mast cell degranulation triggered by activation of PAR-1, because it has been proposed that NO is synthesized in mast cells and modulates stimulated mast cell degranulation accompanied by histamine release, in a cyclic GMP-dependent manner (Masini *et al*., 1991a,b). Involvement of a direct action on the endothelium in the *in vivo* NO modulation of permeability also cannot be excluded, considering that NO decreases the permeability of cultured endothelial monolayers *via* cyclic GMP production under certain conditions (Warren, 1993).

In conclusion, specific activation of PAR-1 induces mast cell degranulation resulting in increased vascular permeability and oedema formation in the rat hindpaw, and this PAR-1-mediated effect is negatively modulated by endogenous and exogenous NO. Based upon the present results and presently available information, we show a hypothetical scheme that explains the roles of PAR-1 and NO in an inflammatory process (Figure 5).

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