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Proteinase-Activated Receptors: Transducers of Proteinase-Mediated Signaling in Inflammation and Immune Response

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Serine proteinases such as thrombin, mast cell tryptase, trypsin, or cathepsin G, for example, are highly active mediators with diverse biological activities. So far, proteinases have been considered to act primarily as degradative enzymes in the extracellular space. However, their biological actions in tissues and cells suggest important roles as a part of the body's hormonal communication system during inflammation and immune response. These effects can be attributed to the activation of a new subfamily of G protein-coupled receptors, termed proteinase-activated receptors (PARs). Four members of the PAR family have been cloned so far. Thus, certain proteinases act as signaling molecules that specifically regulate cells by activating PARs. After stimulation, PARs couple to various G proteins and activate signal transduction pathways resulting in the rapid transcription of genes that are involved in inflammation. For example, PARs are widely expressed by cells involved in immune responses and inflammation, regulate endothelial-leukocyte interactions, and modulate the secretion of inflammatory mediators or neuropeptides. Together, the PAR family necessitates a paradigm shift in thinking about hormone action, to include proteinases as key modulators of biological function. Novel compounds that can modulate PAR function may be potent candidates for the treatment of inflammatory or immune diseases. (*Endocrine Reviews* 26: 1–43, 2005)

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Abbreviations: AP, Activating peptide; AP-1, activator protein-1; $[Ca^{2+}]_i$, intracellular calcium; CGRP, calcitonin-gene related peptide; CNS, central nervous system; COX, cyclooxygenase; $cPLA_2$, cytosolic phospholipase A_2; DRG, dorsal root ganglion; EGF, epidermal growth factor; EGFR, EGF receptor; EPR-1, effector cell proteinase receptor-1; FAK, focal adhesion kinase; GI, gastrointestinal; GM-CSF, granulocyte-macrophage colony-stimulating factor; GP, glycoprotein; GPCR, G protein-coupled receptor; GRK, G protein-coupled receptor kinase; HMEC, human microvascular endothelial cells; hPAR, human PAR; HUVEC, human umbilical vein endothelial cells; ICAM-1, intercellular adhesion molecule-1; IFN-γ, interferon γ; IKK, inhibitor of NFκB kinase; iNOS, inducible NO synthase; JNK, Jun N-terminal kinase; MCP-1, monocyte chemoattractant protein-1; MDCK cells, Madin-Darby canine kidney cells; MMP, matrix metalloproteinase; mPAR, murine PAR; NFκB, nuclear factor κB; NK cells, natural killer cells; NK receptor, neurokinin receptor; NO, nitric oxide; PAF, platelet-activating factor; PAR, proteinase-activated receptor; PAR-AP, proteinase-activated receptor activating peptide; PDGF, platelet-derived growth factor; PG, prostaglandin; PI3, phosphoinositide 3; PKA, protein kinase A; PKB, protein kinase B; PKC, protein kinase C; PLC, phospholipase C; PTX, pertussis toxin; Pyk2, proline-rich tyrosine kinase 2; SLP-76, SH2-domain containing leukocyte-specific phosphoprotein of 76 kDa; SMC, smooth muscle cells; SP, substance P; Src, protein tyrosin kinase Src; TK, tyrosine kinase; VCAM-1 , vascular cell adhesion molecule-1; VSMC, vascular SMC; ZAP-70, ζ-chain-associated protein kinase of 70 kDa.

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I. Introduction

SERINE PROTEINASES CONSTITUTE a family of proteolytic enzymes characterized by a unique catalytic triad consisting of Ser, His, and Asp. These residues are able to hydrolyze peptide bonds (1). Serine proteinases are produced as inactive precursors or zymogens. Subsequent zymogen conversion into a mature physiologically active enzyme is mediated via a process called "limited proteolysis" or zymogen activation (2–4). In mammals, serine proteases, for example, regulate the hemostatic and fibrinolytic balance, degrade neuropeptides involved in neurogenic inflamma-

tion or serve as modulators of immune response during inflammation (5). Three different types of serine proteinase inhibitors can be distinguished based on their mechanism of action: canonical, noncanonical inhibitors, and serpins (6). An imbalance between these inhibitors and their targeted proteinases can affect immune/inflammatory responses and may result in disease. Moreover, the presence of proteinase inhibitors regulates and limits interactions between proteinases and their receptors (7–11).

Recent studies elucidated the ability of certain serine proteinases to regulate cell function via G protein-coupled receptors (GPCRs). At least two different types of proteinase receptors have been identified involving proteolytic cleavage in their activation mechanism: urokinase receptors and proteinase-activated receptors (PARs) (12–14).

PARs belong to a new subfamily of GPCRs with seven transmembrane domains activated via proteolytic cleavage by serine proteinases (13–16). PAR_1, PAR_3, and PAR_4 are targets for thrombin, trypsin, or cathepsin G (17–20). In contrast, PAR_2 is resistant to thrombin, but can be activated by trypsin, mast cell tryptase, factor Xa, acrosin, gingipain, and neuronal serine proteinases (21–28) (Table 1). Interestingly, PARs are activated by a unique mechanism: proteinases activate PARs by proteolytic cleavage within the extracellular N terminus of their receptors, thereby exposing a novel "cryptic" receptor-activating N-terminal sequence that, remaining tethered, binds to and activates the receptor (Fig. 1) within the same receptor (17, 21, 22). Specific residues (about six amino acids) within this tethered ligand domain are believed to interact with extracellular loop 2 and other domains of the cleaved receptor (29), resulting in activation. This intramolecular activation process is followed by coupling to G proteins and the triggering of a variety of downstream signal transduction pathways (see *Sections II.H, III.H,* and *IV.B*; also see Table 2 and Fig. 2, and Refs. 13, 14, and 16). Thus, PARs are not activated like "classical" receptors because the specific receptor-activating ligand is part of the receptor, whereas the circulating agonist is a relatively nonspecific serine proteinase that does not behave like a traditional hormonal regulator akin to insulin.

Several studies during the past few years have also demonstrated that several mechanisms exist to regulate stimulation and termination of PAR-initiated signaling (13–16, 30). Importantly, the availability of PARs at the cell surface is governed by trafficking of the receptor from intracellular stores, and the signaling properties depend on the presence of G proteins and G protein-coupled receptor kinases (GRKs) that modify activity. For PAR_1, PAR_2, and PAR_4, it is well established that short synthetic peptides [PAR-activating peptides (PAR-APs)] designed on their proteolytically revealed tethered ligand sequences can serve as selective receptor agonists (Table 1). Some PAR-APs activate more than one PAR, and they activate receptors at concentrations in the micromolar range as compared with nanomolar potencies of the proteinases themselves (31, 32). Although the PAR_1-AP, SFLLRN-NH_2 also activates PAR_2, PAR_2-APs, like SLIGRL-NH_2 are not capable of activating other PARs. Unfortunately, the relatively low potency (10 to 100 μM EC_{50}) and susceptibility to aminopeptidases (33) limit the utility of the PAR-APs in some bioassay systems. Recently, modified synthetic agonist peptides with higher potency, resistance to aminopeptidases, and greater receptor selectivity have been developed and characterized. These receptor-selective agonists are of use to study the consequences of activating PARs *in vivo* (Table 1) (13, 14, 16, 34). So far, antagonists

TABLE 1. Pharmacology of proteinase-activated receptors and their agonists

	PAR_1	PAR_2	PAR_3	PAR_4
Amino acid composition	425 aa (h)	397 aa (h)	374 aa (h)	385 aa (h)
Tethered ligand sequence	SFLLR (h) SFFLR (m, r)	SLIGKV (h) SLIGRL (m, r)	TFRGAP (h) SFNGGP (m)	GYPGQV (h) GYPGKV (m)
Selective agonist peptide	TFLLR-NH_2 TFRIFD[a]	SLIGKV-NH_2 (h) SLIGRL-NH_2 (m, r)	None known	GYPGKV-NH_2 GYPGQV-NH_2 AYPGKF-NH_2
High-affinity ligands	Thrombin	Trypsin, tryptase, trypsin-2	Thrombin[b]	Thrombin, trypsin
Low-affinity ligands	Trypsin; FVIIa/TF/FXa; granzyme A, plasmin	Matriptase/MT-serine protease 1; Der p3; Der p9; FVIIa/TF/FXa; cockroach proteases	Trypsin > factor Xa	Cathepsin G, factors VIIa/X
Inhibitory proteases	Cathepsin G, proteinase-3; elastase; chymase	Elastase, chymase	Cathepsin G	Unknown
Selective antagonists	Trans-cinnamoyl-parafluoro-Phe-Paraguanidino-Phe-Leu-Arg-Arg-NH_2 Mercaptopropionyl-Phe-Cha-Arg-Lys-Pro-Lys-Pro-Asn-Asp-Lys-NH_2 Non-peptide antagonists: RWJ56110 and RWJ58259	None known	None known	Trans-cinnamoyl-YPGKF-NH_2

The letters denote the amino acid sequences of peptides in one letter code: aa, amino acids; h, human; m, mouse; r, rat; NH_2, respective amides.

[a] TFRIFD is the *Xenopus* thrombin receptor tethered ligand domain (the human PAR_1 tethered ligand domain sequence, SFLLRN, activates both PAR_1 and PAR_2).

[b] Proteinase cleavage but PAR_3 does not generate a calcium signal.

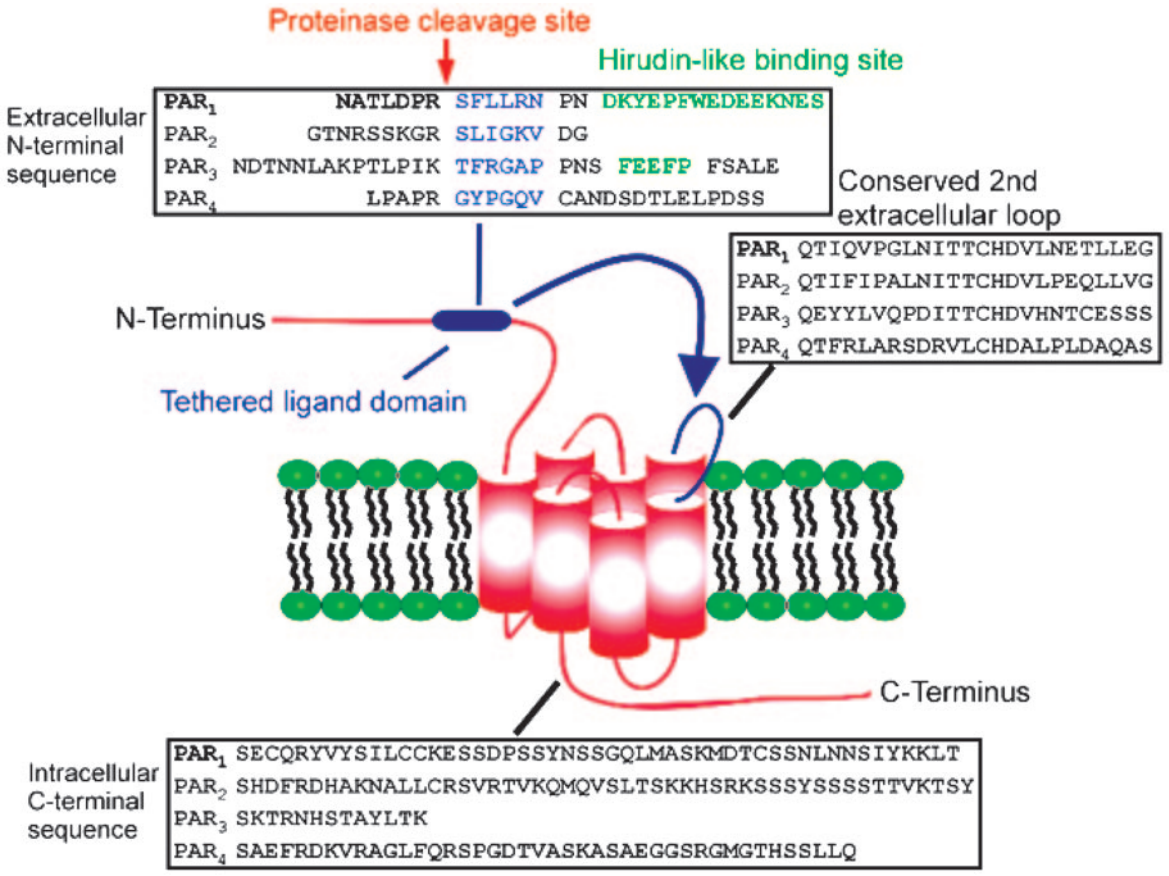

FIG. 1. Classical mechanism of PAR activation and their structural features. The proteinase cleaves the PAR receptor at a particular site (marked by *red arrow*), unmasking a previously cryptic N-terminal sequence of the receptor (presented in *blue*), defined as "tethered ligand." This tethered ligand sequence interacts with the conserved second extracellular loop and activates the same receptor. The human sequences of the tethered ligand, the second extracellular loop, and the intracellular C-terminus are presented in the *boxes*. Hirudin-like binding domain sequences for PAR_1 and PAR_3 are printed in *green*.

for PAR_1 (35–37) and PAR_4 (38) have been synthesized, but are not yet available for PAR_2. PAR_3, as will be elaborated upon in *Section IV*, remains a puzzle, in that studies with the murine receptor indicate that it cannot be activated either by its cognate synthetic tethered ligand peptide or by thrombin (39, 40). Rather than acting as an independent cell regulator, PAR_3 appears to function as a cofactor for the activation of PAR_4 (39). Complementary data documenting PAR-mediated effects in various tissues have been obtained using PAR-deficient ($PAR^{-/-}$) mice.

Taken together, data obtained using the enzyme activators themselves (trypsin, thrombin), the PAR-APs and using PAR gene-deficient mice provide compelling evidence that PARs play a critical role in the regulation of various physiological and pathophysiological functions in mammals, including humans. This review focuses on the biology and signaling properties of PARs in various mammalian tissues and highlights the current knowledge about the role of PARs during inflammation and immune response. To complement the information summarized in the sections that follow, the reader is encouraged to consult a number of other recent reviews concerning the activation mechanisms and cell biological aspects of PARs (13, 14, 16, 34, 41–45).

II. PAR_1 in Inflammation and Immune Response

Thrombin is an important effector proteinase of the coagulation cascade that leads to formation of a hemostatic plug. Thrombin is thought to act near the site at which it is generated and it is activated when circulating coagulation factors in the blood plasma make contact with tissue factor. Tissue factor is a membrane protein that is usually produced by cells that are separated from blood (*i.e.*, epithelial cells). However, it is also expressed at low levels on circulating monocytes and microparticles from leukocytes. Tissue factor is associated with the activation of zymogen factor X by factor VIIa. Factor Xa together with its cofactor Va subsequently converts prothrombin to the active enzyme. Thus, plasma coagulation can only take place when the vascular integrity is damaged (15). Thrombin causes shape change of endothelial cells and increased permeability of endothelial cell layers.

However, recent evidence revealed that thrombin is not only a clotting proteinase serving as both a pro- and anticoagulant molecule but also appears to play multifunctional roles related to inflammation, allergy, tumor growth, metastasis, tissue remodeling, thrombosis, and probably wound healing (13, 15, 42, 43). Subsequent to the cloning of PAR_1 (17, 46), it was realized that many of the cellular actions of throm-

TABLE 2. Biology and pathophysiology of PAR activation in different tissues, cells, and species

Tissue	Cell type	Effect	Refs.
		PAR_1-agonist mediated effects	
Airways (Chinese hamster)	CCL39, PS200	G_i, PI hydrolysis, $pp60^{src}$, ERK1/2, SHC/Grb-2 kinase, Src/Ras, Fyn, adenylyl cyclase inhibition, growth, mitogenesis	Hung *et al.* (115) Chen *et al.* (349, 350) Trejo *et al.* (351)
Airways ($PAR_1^{-/-}$ mouse)	Fibroblasts	$[Ca^{2+}]_i$ ↑, PTX-(in)sensitive and PKC/c-Raf-(in)dependent → MAPK, mitogenesis	Connolly *et al.* (135) Trejo *et al.* (351)
Airways (guinea pig)	Lung, parenchymal strips	Contraction	Saifeddine *et al.* (159)
Airways (human)	HFL1, adult lung fibroblasts	CTGF ↑	Chambers *et al.* (148)
Airways (human)	HBEC, A459, BEAS-2B	$[Ca^{2+}]_i$ ↑, IL-6 ↑, IL-8 ↑, PGE_2 ↑	Asokananthan *et al.* (77)
Airways (mouse)	Tracheal SMC	PGE_2 ↑, relaxation/contraction	Lan *et al.* (152, 153)
Airways (rat)	Epithelium, trachea, bronchi	Contraction	Chow *et al.* (91)
Blood (human)	Platelets	AP: integrin-α(IIb)β(3) ↓, Src ↓, FAK ↓, SHP-1 phosphorylation; thrombin: calpain ↑, Src, FAK, and integrin $\alpha_{IIb}\beta_3$ not effected; cytoskeletal reorganization, disaggregation	Ramars *et al.* (352)
Blood (human)	Platelets	$[Ca^{2+}]_i$ ↑, PLC, PI_3P, $pp72^{Syk}$, p54/58lynv, p60c-src, annexin V binding, platelet aggregation (ADP and MMP-2 dependent) and activation	Vu *et al.* (17) Huang *et al.* (353) Hung *et al.* (326) Hwa *et al.* (248) Andersen *et al.* (122) Kahn *et al.* (128) Chung *et al.* (334)
Blood (human)	Platelets	Degranulation, TXA_2/TXB_2 ↑	Henriksen *et al.* (354) Henriksen and Hanks (117) McRedmond *et al.* (133)
Blood (human)	Platelets	PI3 kinase, PKC, Rho$\beta\gamma$-subunits, Pyk-2, FAK, Src, adenylyl cyclase inhibition, aggregation	Zhang *et al.* (204) Ohmori *et al.* (203, 355) Kim *et al.* (201)
Blood (human)	Platelets	IVSn-14A/T intronic gene-variation associated to PAR_1 surface density, different agonist response	Dupont *et al.* (127)
Blood (human)	Platelets	$p160^{ROCK}$-mediated shape change; 1) $G_{12/13}/Ca^{2+}$-mediated; 2) G_i/PI3 kinase-mediated GPIIb/IIIa activation	Dorsam *et al.* (356)
Blood (human)	Platelets	Induced clearance of cell surface GPIb	Han *et al.* (357)
Blood vessel (human)	HUVEC	$G_{\beta\gamma}$/p85/p110/PI3 kinase and $G_{\alpha q}$/PKC-δ → Akt → NFκB → ICAM-1 ↑ Rho A, WPB exocytosis, permeability ↑	Rahman *et al.* (228, 231, 232) Chi *et al.* (72) Klarenbach *et al.* (358)
Blood vessel (human)	HUVEC	APC treatment as well as PAR_1-AP induces MCP-1 expression	Riewald *et al.* (359)
Blood vessel (human)	HUVEC, HMEC	$cPLA_2\alpha$, PGI_2 ↑; shear stress → PAR_1 ↓; TNF-α → PAR_1 ↓	Nguyen *et al.* (101) Shinohara *et al.* (102)
Blood vessel (porcine)	Coronary artery	Endothelium-dependent relaxation	Hwa *et al.* (248)
Bone (human)	Synovial fibroblasts	RANTES mRNA ↑	Hirano *et al.* (80)
Brain (human)	Brain microvascular endothelial cells, HUVEC	APC → $[Ca^{2+}]_i$ ↑ via EPCR and PAR_1; cyto/neuroprotection	Domotor *et al.* (360) Cheng *et al.* (361)

TABLE 2. *Continued*

Tissue	Cell type	Effect	Refs.
Brain (mouse)	Astrocytes	Hsp90 binds PAR_1, affects cytoskeleton, PKC β-1 translocation	Pindon *et al.* (190) Pai *et al.* (362)
Brain (rat)	Astrocytes	$[Ca^{2+}]_i$ ↑, proliferation, ERK-1/2-activation via 1) PI3-kinase 2) G_q-PLC-Ca^{2+}-PKC Pyk2/Grb2/Src	Ubl *et al.* (176, 363) Wang *et al.* (185) Wang and Reiser (210, 466)
Brain (rat)	Astrocytes	Proliferation, reversion of stellation; creatine kinase interacts with PAR_1 → RhoA → cytoskeletal reorganization	Debeir *et al.* (191) Mahajan *et al.* (364)
Breast (human)	MDAMB231	$[Ca^{2+}]_i$ ↑, migration/invasion (G_i/PI3-kinase-mediated) ↓	Kamath *et al.* (365)
Cardiovascular system (porcine)	Artery	Relaxation	Hwa *et al.* (248)
Cardiovascular system (rat)	Aortic arterial ring	Relaxation	Hollenberg *et al.* (158) Muramatsu *et al.* (64)
Cardiovascular system (rat)	Fibroblasts, cardiomyocytes	$[Ca^{2+}]_i$ ↑, Src ↑, Fyn ↑, EGFR transactivation, ERK-1/2 and p38 phosphorylation	Sabri *et al.* (366)
Cardiovascular system (rat/mouse)	NRVM, cardiomyocytes	$[Ca^{2+}]_i$ ↑, IP accumulation, EGFR kinase, activation of ERK-1/2 and p38, c-Jun, Akt, ANF, hypertrophy	Steinberg *et al.* (367) Jiang *et al.* (368) Sabri *et al.* (222, 338)
Cardiovascular system (rat)	RASMC	$[Ca^{2+}]_i$ ↑	Takada *et al.* (76)
Endometrium (human)	Endometrial stromal cells	Levonorgestrel → PAR_1 ↓	Hague *et al.* (369)
Gallbladder (guinea pig)	SMC	Prostanoid-mediated contraction	Tognetto *et al.* (370)
GI tract (rat)	Esophageal smooth muscle	Contraction, Na^+ influx, $[Ca^{2+}]_{ex}$– influx (PAR_4 opposed)	Kawabata *et al.* (371)
Immune system (human)	Alveolar macrophages	PAR_1 ↑ in AMs of smokers	Roche *et al.* (372)
Immune system (rat)	BMCMC	Fibronectin adhesion via (PTX)-sensitive G_i-protein, PI3 kinase, PKC, activation of ERK-1/2 and p38, IL-6 ↑, MMP-9 ↑, β-hexosaminidase ↑	Vliagoftis *et al.* (373)
Immune system (rat)	Mast cells	Degranulation ↑, degranolysis ↑, heparin release	Umarova *et al.* (374)
Immune system (human)	Monocytes, macrophages	$[Ca^{2+}]_i$ ↑, MCP-1	Colognato *et al.* (375)
Intestine (guinea pig)	Longitudinal smooth muscle	Contraction, dependent on $[Ca^{2+}]_{ex}$, TK, COX	Hollenberg *et al.* (158) Saifeddine *et al.* (159)
Intestine (guinea pig)	Taenia coli	Relaxation	Cocks *et al.* (376)
Intestine (human)	Colonic epithelial cells	Cellular invasion via 1) $G\alpha_{12}$/$G\alpha_{13}$-RhoA/RhoD/ROCK 2) $G\alpha_q$, PLCβ, CaMLCK, MLC-phosphorylation	Nguyen *et al.* (377)
Intestine (human)	SCBN cells	$[Ca^{2+}]_i$ ↑, Cl^- secretion via MEK-ERK/MAPK-cPLA$_2$, involves Src, EGFR and COX-1/2 activity	Buresi *et al.* (156, 378)
Intestine (mouse)	Model, smooth muscle	Modulates GI transit, L-type Ca^{2+} channel, contraction, PI3 kinase, PLC, PKC, TK	Kawabata *et al.* (161, 379)
Intestine (mouse)	Gastric fundus	Relaxation/contraction	Cocks *et al.* (376)
Intestine (rat)	Longitudinal and circular muscle	Biphasic contraction, relaxation	Mule *et al.* (163)
Intestine (rat)	SMC, vascular muscle cells	Irradiation → PAR_1 ↑	Wang *et al.* (164)

TABLE 2. *Continued*

Tissue	Cell type	Effect	Refs.
Kidney (murine)	Model	Renal inflammation	Cunningham *et al.* (49)
Lymph node (human)	LNCaP	RhoA, cytoskeletal reorganization	Greenberg *et al.* (380)
Muscle (mouse)	Neonatal myoblasts	$[Ca^{2+}]_i$ ↑, proliferation	de Niese *et al.* (381)
Nervous system/intestine (guinea pig)	Enteric neurons	Excitatory response, dependent on PLC and $[Ca^{2+}]_i$	Gao *et al.* (319)
Nervous system (guinea pig)	Myenteric glia	$[Ca^{2+}]_i$ ↑, PLC	Garrido *et al.* (382)
Nervous system (guinea pig)	Myenteric neurons	$[Ca^{2+}]_i$ ↑	Corvera *et al.* (196)
Nervous system (CNS) (human)	A172	$[Ca^{2+}]_i$ ↑, PKC-α, PKC-γ, PKC-L	Okamoto *et al.* (317)
Nervous system (human)	Neuronal cells	MMP-1 potentiates PAR_1 induced ↑ $[Ca^{2+}]_i$ release	Conant *et al.* (383)
Nervous system ($PAR^{-/-}$ mouse)	Model	Less PAR_1 → posttraumatic survival of CNS neurons ↑	Friedmann *et al.* (186)
Nervous system CNS ($PAR^{-/-}$ mouse)	Primary microglial cells	Activation of p38, p44/42, CD40 ↑, proliferation	Suo *et al.* (83)
Nervous system CNS (human)	Hippocampal neurons	PAR_1 activation potentiates NMDA receptor activity	Fang *et al.* (384)
Nervous system (rat)	Primary afferent neurons	PAR_1-AP: mechanical and thermal analgesia, carrageenan-induced mechanical and thermal hyperalgesia ↓; thrombin: carrageenan-induced mechanical analgesia ↓	Asfaha *et al.* (197) Kawabata *et al.* (198)
Nervous system (rat)	Sensory nerves	Plasma extravasation, edema, SP ↑ → NK_1 receptor activation	de Garavilla *et al.* (155)
Pancreas (human)	MIA PaCa-2	Adhesion to extracellular matrix-proteins ↑ (laminin, collagen IV, fibronectin); PAR_1 ↑ in pancreatic cancer	Rudroff *et al.* (165, 166)
Skin (human)	HDMEC, HMEC, HPAEC	$G_{\alpha i}$-linked Src-Ras-MAPK-pathway activation. PI3 kinase, PAR_1 expression	Ellis *et al.* (385)
Skin (oral mucosa) (human)	KB cells	$[Ca^{2+}]_i$ ↑, IL-6 ↑, (RgpB)-induced	Lourbakos *et al.* (28)
Skin (human)	Keratinocytes	$[Ca^{2+}]_i$ ↑, proliferation ↑, inhibits differentiation	Derian *et al.* (275) Algermissen *et al.* (386)
Skin (human)	SIT1	High TF level and activated PAR_1 → enhanced metastasis	Bromberg *et al.* (387)
Skin ($PAR_1^{-/-}$ mouse)	Fibroblasts, model	Plasmin activates PAR_1 → ERK-1/2 → Cyr61 ↑. Sepsis protection, APC activates EPCR/PAR_1 receptor cascade, MCP-1; 1) G_q → PKC → c-Raf → MAPK 2) G_i → MAPK	Trejo *et al.* (351) Pendurthi *et al.* (388) Riewald *et al.* (81)
Vascular system (human)	HASMC	Cyclic strain → PAR_1 ↑ Low shear stress → PAR_1 ↑ High shear stress → PAR_1 ↓	Papadaki *et al.* (389) Nguyen *et al.* (104)
Vascular system (human)	HCASMC, HASMC, HAOAF, HAEC	FXa-induced: proliferation, ↑ IL-6, ↑ prothrombotic TF	McLean *et al.* (86)
Vascular system (human)	Pulmonary artery	Endothelium-dependent relaxation	Hamilton *et al.* (99)
Vascular system ($PAR_1^{-/-}$ mice)	Model	Embryonic development; vascular development, endothelium-specific mPAR_1 expression → reduction of embryonic death	Connolly *et al.* (135) Griffin *et al.* (96)
Cell line (Chinese hamster)	CHO cells	PAR_1 third intracellular loop: G_i coupling, MAPK activation, internalization; PAR_1 C-tail: internalization, PLC coupling	Chen *et al.* (390, 391)

TABLE 2. *Continued*

Tissue	Cell type	Effect	Refs.
Cell line (Chinese hamster)	CHO/TF	Ternary complex TF-VII-Xa activates PAR_1	Riewald and Ruf (241)
Cell line (human)	HeLa	FXa → gene induction, ERK1/2, Cyr61, CTGF	Riewald *et al.* (239)
Cell line (monkey)	COS-7 cells	G_{12}, G_{13}, G_q, PI hydrolysis, ERK-5	Shapiro *et al.* (392) Verrall *et al.* (29) Fukuhara *et al.* (393)
In vitro assay	*In vitro* assay	Noncoding RNA (ncR-uPAR) regulates PAR_1 expression during embryogenesis	Madamanchi *et al.* (394)
HT29	Human colon cancer cell line	Mitogenic activity up-regulated; p42/p44 activation	Darmoul *et al.* (395)
Model (cynomogul monkey)	Model, platelets	RWJ-58259 → blood flow ↑; prevents thrombosis and vascular occlusion	Derian *et al.* (107)
		Thrombin-mediated effects	
Airways (bovine)	Tracheal SMC	PI3 kinase, PKB, PI_3P	Walker *et al.* (145)
Airways (Chinese hamster)	CCL 39 fibroblasts, PS200, Rat-1)	$p21^{RAS}/G_i/G_o$, p70s6k, p44	Kahan *et al.* (396) van Corven *et al.* (397)
Airways (guinea pig)	Trachea, main bronchi	Bronchoconstriction, biphasic change in arterial blood pressure	Cicala *et al.* (151) Carr *et al.* (303)
Airways (human)	ASM	$[Ca^{2+}]_i$ ↑, PI3 kinase ↑, $pp70^{s6k}$, proliferation; thrombin → GM-CSF ↑ independent of PAR_1	Krymskaya *et al.* (223) Tran and Stewart (398)
Airways (human)	ASM, bronchial rings	Contraction	Hauck *et al.* (150)
Airways (human)	Fetal lung fibroblast	α1(I)Procollagen ↑	Chambers *et al.* (146)
Airways (human)	BEAS-2B, BSMC, NHBE	PDGF-AB ↑	Shimizu *et al.* (149)
Airways (mouse)	Tracheal SMC	PGE_2 ↑, relaxation/contraction	Lan *et al.* (152, 153)
Airways (rat)	Epithelium, trachea, bronchi	Contraction	Chow *et al.* (91)
Blood vessel (human)	HUVEC, primary umbilical cords	$[Ca^{2+}]_i$ ↑, Il-6 ↑, PLC, PKC, PGI_2, PDGF mRNA ↑, vWf release, P-selectin ↑, neutrophil adhesion, barrier dysfunction, contraction	Sugama *et al.* (47) Garcia *et al.* (67) Storck and Zimmermann (399) Chi *et al.* (72)
Blood vessel (bovine)	Pulmonary artery	G_i, $[Ca^{2+}]_i$ ↑, PLC, PKC, PI3 kinase, PGI_2 ↑, PDGF, $p70^{s6k}$, growth, contraction, permeability	Tiruppathi *et al.* (400) Garcia *et al.* (67) Belham *et al.* (401)
Blood vessel (bovine)	VSMC	$[Ca^{2+}]_i$ ↑, IL-6 ↑, MCP-1 ↑	Kranzhofer *et al.* (402) Bretschneider *et al.* (321)
Blood vessel (human)	HUVEC, HMEC	$[Ca^{2+}]_i$-dependent Pyk-2 activation (PKC, Src kinases independent); $cPLA_2\alpha$, COX-2 ↑, PGI_2 ↑; Shear stress → PAR_1 ↓; TNF-α → PAR_1 ↓	Tiruppathi *et al.* (400) Nguyen *et al.* (101) Houliston *et al.* (78) Keogh *et al.* (403) Shinohara *et al.* (102)
Blood vessel (porcine)	Pulmonary artery SMC	$[Ca^{2+}]_i$ ↑	Tiruppathi *et al.* (400)
Bone (rat)	Primary osteoblast-like cells, Saos-2 cells	$[Ca^{2+}]_i$ ↑, alkaline phosphatase activity ↓; TGF-β, thrombin → PAR_1 ↑; PDGF-BB → PAR_1 mRNA ↓	Jenkins *et al.* (404) Abraham and Mackie (405)
Bone (human)	VcaP	PAR_1 ↑, $[Ca^{2+}]_i$ ↑	Chay *et al.* (406)
Brain (human)	1321N1 astrocytoma cells	$p21^{RAS}$, AP-1, mitogenesis; cell rounding, GEF/Rho/G_{12}/G_{13}	Majumdar *et al.* (213) LaMorte *et al.* (214)
Brain (human)	Meningioma cells	$[Ca^{2+}]_i$ ↑	Kaufmann *et al.* (407)

TABLE 2. *Continued*

Tissue	Cell type	Effect	Refs.
Brain (human)	SNB-19 glioblastoma cells	Meizothrombin, thrombin and SFLLRN → $[Ca^{2+}]_i$ ↑, PKA activation, NFκB-associated translocation of PKAα into nucleus, cAMP independent	Kaufmann *et al.* (328) Zieger *et al.* (217)
Brain (rat)	C6 glioma cells	$[Ca^{2+}]_i$ ↑, iNOS ↑	Ubl *et al.* (176) Meli *et al.* (85)
Brain (rat)	Cholinergic neuron	Modulation of cholin acyltransferase activity	Debeir *et al.* (408)
Brain (rat)	RBCE	$[Ca^{2+}]_i$ ↑	Domotor *et al.* (302)
Cardiovascular system (rat)	RASMC	$[Ca^{2+}]_i$ ↑ Rho, Rho kinase, migration, proliferation	McNamara *et al.* (409) Chaikof *et al.* (410) Seasholtz *et al.* (219)
Circulatory system (rat)	Model	Biphasic change in MABP, hypertension (NO and nerval modulated)	Cicala *et al.* (411)
Connective tissue (human)	Synovial fibroblasts	Proliferation, IL-6 ↑, GM-CSF	Shin *et al.* (143, 412)
Eye (human)	HCEC (human corneal epithelial cells)	$[Ca^{2+}]_i$ ↑, IL-6, IL-8, TNF-α	Lang *et al.* (339)
Immune system (human)	Monocytes (U 937), PBMC (IFN-γ-differentiated)	IL-1α ↑, IL-1β ↑, $p21^{CIP1/WAF1}$ ↓, cyclin D_1 ↑ → proliferation; thrombin: IL-6 ↑, TNF-α ↑, PAR_1 ↑	Naldini *et al.* (137, 141, 413)
Immune system (human)	Lymphocytes	CD69 ↑, IL-2 ↑	Mari *et al.* (235)
Immune system (human)	Jurkat T-leukemic cells	$[Ca^{2+}]_i$ ↑, PKC ↑, CD69 ↑, IL-2 ↑, Vav1, ZAP-70, SLP-76	Mari *et al.* (235) Bar-Shavit *et al.* (236)
Immune system (rat)	Peritoneal mast cells	NO ↑, PAF ↓	Strukova *et al.* (414)
Intestine (guinea pig)	Longitudinal smooth muscle	Contraction, dependent on $[Ca^{2+}]_{ex}$, COX	Hollenberg *et al.* (158) Saifeddine *et al.* (159)
Intestine (guinea pig)	Taenia coli	Relaxation	Cocks *et al.* (376)
Intestine (mouse)	Gastric fundus	Relaxation/contraction	Cocks *et al.* (376)
Kidney (canine) and intestine (human)	MDCK, MDCKts.src, colonic PCmsrc epithelial cells, HCT-8/S11	$PAR_1/G\alpha_i$ inhibit TFFs-induced invasion; $PAR_1/G\alpha_{olf}$ inhibit invasion	Faivre *et al.* (168) Regnauld *et al.* (169)
Kidney (human)	Glomerular mesangial cells	$pp125^{FAK}$	Choudhury *et al.* (415)
Kidney (rat)	GEC, GMC	Clusterin mRNA ↑, TGF-β ↑	Laping *et al.* (416)
Liver (human)	HepG2	PKC, JNK/p38 MAPK	Mitsui *et al.* (417)
Monocytes (human)	Mononuclear cells	IL-1 ↑, IL-6 ↑	Naldini *et al.* (82)
Nervous system (chicken)	Motoneurons	Neuronal apoptosis, caspases-1 and -3	Turgeon *et al.* (418)
Nervous system/intestine (guinea pig)	Enteric neurons	Excitatory response, dependent on PLC and $[Ca^{2+}]_i$	Gao *et al.* (319)
Nervous system (mouse)	NB2a	Morphological changes	Pai and Cunningham (419)
Skeletal muscle (human)	Myocytes	$[Ca^{2+}]_i$ ↑	Mbebi *et al.* (420)
Skin (human)	HMEC	GRK5 → PAR_1 phosphorylation, PAR_1-mediated $[Ca^{2+}]_i$ increase ↓	Tiruppathi *et al.* (105)
Skin (human)	Keratinocytes	$[Ca^{2+}]_i$ ↑, proliferation, inhibits differentiation	Derian *et al.* (275) Algermissen *et al.* (386)
Skin (human)	Primary FS4 fibroblasts, prostate DU145, CHRF	CVEGF, PI3 kinase, serine/threonine kinase	Huang *et al.* (421)

TABLE 2. *Continued*

Tissue	Cell type	Effect	Refs.
Skin (mouse)	Fibroblasts	Fibroblast/macrophage ratio ↑, fibroblast proliferation, epithelial and endothelial cells ↑, neovascularization → wound healing ↑	Strukova *et al.* (414)
Skin (mouse)	NIH-3T3 fibroblasts	$G\alpha_{12}/G\alpha_{13}$, RAF-1, TK, ERK1/2, RhoA-mediated transformation	Apostolidis and Weiss (422) Martin *et al.* (90)
Urogenital tract (human)	RT4, HUR	AA ↑, PGE_2 release, $iPLA_2$, PAF, phospholipid hydrolysis	McHowat *et al.* (170) Rickard and McHowat (423)
Vascular system (guinea pig)	Carotid artery	Biphasic change in arterial blood pressure (hypertension/ hypotension)	Cicala *et al.* (151)
Vascular system (human)	VSMC	$[Ca^{2+}]_i$ ↑, ERK-1/2 activation, mitogenesis	Bretschneider *et al.* (220)
Vascular system (rat)	VSMC	p38 MAPK, cAMP-response element promotor dependent transcription, growth	Ghosh *et al.* (221)
Vascular system (rat)	VSMC	Progesteron, gestodene → PAR_1 ↑ → TF-expression ↑, procoagulant activity ↑	Herkert *et al.* (68)
Vascular system (rat)	VSMC	α1(I)procollagen (mRNA) ↑	Dabbagh *et al.* (100)
Cell line (human)	CHRF-288	Blebbing, PI3 kinase	Vemuri *et al.* (424)
		PAR_2-agonist mediated effects	
Airways (guinea pig)	Epithelial cells, SMC	Bronchoconstriction/bronchorelaxation; trypsin but not AP caused	Ricciardolo *et al.* (290)
Airways (guinea pig)	Tracheal epithelial cells	$[Ca^{2+}]_i$ ↑	Oshiro *et al.* (425)
Airways (guinea pig)	Trachea, upper bronchi	Bronchoprotection to histamine	Cicala *et al.* (293)
Airways (guinea pig)	Tracheal rings	Trypsin: contraction, NK-receptor involved; AP has no effect	Carr *et al.* (303)
Airways (human)	A549	$[Ca^{2+}]_i$ ↑, proliferation, GM-CSF ↑, eotaxin ↑, PI hydrolysis, Der p3 and Der p9 → PLC activation; MMP-9 release, MEK-1, MAPK, NFκB	Böhm *et al.* (154) Vliagoftis *et al.* (297) Sun *et al.* (343)
Airways (human)	A549, HBE	Thermolysin → $[Ca^{2+}]_i$ ↑	Ubl *et al.* (296)
Airways (human)	Bronchial epithelial cells	Inhibits ion transport (basolateral K^+-permeability ↓)	Danahay *et al.* (426)
Airways (human)	Bronchial SMC	$[Ca^{2+}]_i$ ↑, contraction, contributes to allergic inflammation and immunity	Schmidlin *et al.* (291)
Airways (human)	Epithelial cells	PAR_2 in asthmatic tissue ↑	Knight *et al.* (427)
Airways (human)	Fibroblasts	Proliferation	Akers *et al.* (428)
Airways (human)	HASMC	$[Ca^{2+}]_i$ ↑, histamine-mediated contraction ↑	Chambers *et al.* (294)
Airways (human)	HBEC, A459, BEAS-2B	$[Ca^{2+}]_i$ ↑, IL-6 ↑, IL-8 ↑, PGE_2 ↑	Asokananthan *et al.* (77)
Airways (human)	SAECs, A549	GM-CSF ↑, eotaxin ↑	Vliagoftis *et al.* (272)
Airways (human)	SMC	Proliferation, via a PTX-sensitive G protein, $[Ca^{2+}]_i$ ↑, PKC, PLC, IP_3	Berger *et al.* (318, 320)
Airways (human, guinea pig, rat, mouse)	Bronchial, epithelial (isolated airway)	COX-dependent bronchorelaxation, bronchoconstriction inhibited	Cocks *et al.* (292)
Airways (mouse)	Model	Inhibits neutrophil recruitment; eosinophil recruitment, hyperreactivity, allergic inflammation	Moffatt *et al.* (270) Schmidlin *et al.* (271)

TABLE 2. *Continued*

Tissue	Cell type	Effect	Refs.
Airways (mouse)	Tracheal SMC	Relaxation, PGE_2 ↑, COX-2, E-type prostanoid-2 receptor activation	Lan *et al.* (152, 153)
Airways (rat)	Epithelium, trachea, bronchi	Contraction, epithelium-dependent relaxation	Chow *et al.* (91)
Airways (guinea pig)	Epithelium trachea	Reduction of response to LPS, potentially mediated via PKC-ε and PKC-α	Oshiro *et al.* (429)
Blood (human)	Platelets	$[Ca^{2+}]_i$ ↑, platelet aggregation, (RgpB)-induced	Lourbakos *et al.* (329)
Blood vessels (human)	HUVEC	$[Ca^{2+}]_i$ ↑, IL-6 ↑, COX-2 ↑, PGI_2 ↑; Rho A, WPD exocytosis	Chi *et al.* (72) Houliston *et al.* (78) Klarenbach *et al.* (358)
Blood vessels (human)	HUVEC	FXa → $[Ca^{2+}]_i$ ↑, EPR-1 dependent	Bono *et al.* (322)
Blood vessels (human)	Model	Vasodilatation	Robin *et al.* (430)
Blood vessels (human)	Vascular wall cells (HAoAF, HCSMC, HASMC)	FXa: proliferation, $[Ca^{2+}]_i$ ↑, IL-6 ↑, prothrombotic TF ↑; TF/FVIIa-induced cell migration	McLean *et al.* (86) Marutsuka *et al.* (431)
Blood vessel (mouse)	Isolated mesenteric arteriole	Relaxation (NO-cGMP-dependent and independent)	McGuire *et al.* (432)
Blood vessels (mouse)	Mouse model of hindlimb ischemia	Angiogenesis, hemodynamic recovery	Milia *et al.* (433)
Blood vessels (rat)	Aorta and jugular veins	LPS → PAR_2 ↑, regulation of blood pressure, relaxation via endothelium and NO-dependent mechanism	Hwa *et al.* (248) Cicala *et al.* (434) Compton *et al.* (259)
Bone (rat)	Osteoblasts	$[Ca^{2+}]_i$ ↑	Abraham *et al.* (254)
Brain (human)	Meningioma cells	$[Ca^{2+}]_i$ ↑	Kaufmann *et al.* (301)
Brain (human)	CCF-STTG1	TNF-α secretion; trypsin: ERK, p38	Kim *et al.* (435)
Brain (rat)	Astrocytes	$[Ca^{2+}]_i$, proliferation	Wang *et al.* (185)
Brain (rat)	RBCE	$[Ca^{2+}]_i$ ↑	Domotor *et al.* (302)
Cardiovascular system (bovine)	VSMC	$[Ca^{2+}]_i$ ↑, NFκB ↑	Bretschneider *et al.* (321)
Cardiovascular system (human)	CASM	FXa → ERK-1/2 phosphorylation, mitogenesis	Koo *et al.* (323)
Cardiovascular system (human)	HUVEC, endothelial cultures	$[Ca^{2+}]_i$ ↑, vWf release, mitogenesis	Mirza *et al.* (97) Storck *et al.* (266)
Cardiovascular system (human)	SMC	Mitogenesis, PDGF release	Bono *et al.* (436)
Cardiovascular system (rat)	Endothelium (aortic rings)	Relaxation, NO ↑	Kawabata *et al.* (437)
Cardiovascular system (rat)	Neonatal ventricular cardiomyocytes	$[Ca^{2+}]_i$ ↑, IP_3, ERK-1/2, p38, hypertrophy	Sabri *et al.* (222)
Cardiovascular system (rat)	SMC	Relaxation/contraction	Al Ani *et al.* (246) Saifeddine *et al.* (250) Emilsson *et al.* (438) Roy *et al.* (439)
Circulatory system (rat)	Model	Biphasic change in MABP, NO and nerval-modulated hypertension, NO-modulated hypotension	Cicala *et al.* (411)
Exocrine glands (mouse and rat)	Salivary, parotid, sublingual	Glandular exocrine secretion, saliva production, amylase secretion, mucin secretion (via TK activation, partially NO involved)	Kawabata *et al.* (310, 311, 440)

TABLE 2. *Continued*

Tissue	Cell type	Effect	Refs.
Gallbladder (guinea pig)	SMC	Prostanoid-mediated contraction	Tognetto *et al.* (370)
GI tract (human)	MKN-1	Integrin $\alpha_5\beta_1$-dependent adhesion to fibronectin (high) and vitronectin (low), G_i-protein, Src kinase, proliferation	Miyata *et al.* (441)
GI (guinea pig)	Pepsinogen-secreting (chief) cells	Stimulation of pepsinogen secretion	Fiorucci *et al.* (442)
GI tract (rat)	Gastric SMC	Contraction	Al Ani *et al.* (246) Saifeddine *et al.* (250)
GI tract (rat)	Intestinal-neurogenic mechanism	Mucus secretion ↑, cytoprotection (via CGRP1 and NK_2 stimulation, triggered by CGRP tachykinin release from sensory neurons)	Kawabata *et al.* (312)
GI (human)	Colon mast cells	Up-regulation of tryptase and histamine release	He *et al.* (443)
GI tract (rat)	Model approach	Acid secretion suppressed (independently of prostanoid production and sensory neurons)	Nishikawa *et al.* (444)
Heart (rat)	Isolated	Inflammation ↓, neutrophil recruitment ↓, endothelium-dependent coronary vasodilation, EDHF ↑, vanilloid receptor-1 activation, c-fibers involved	Napoli *et al.* (345) McLean *et al.* (445)
Immune system (human)	Eosinophils	Superoxide production, degranulation	Miike *et al.* (267)
Immune system (human)	Jurkat T-leukemic cell line	Vav1, ZAP-70, SLP-76	Bar-Shavit *et al.* (236)
Immune system (human)	Mononuclear cells	Cathepsin G → $[Ca^{2+}]_i$ ↑	Roche *et al.* (372)
Immune system (human)	Monocytes, macrophages	$[Ca^{2+}]_i$ ↑	Colognato *et al.* (375)
Immune system (human)	Neutrophils	Gingipain-R → $[Ca^{2+}]_i$ ↑	Lourbakos *et al.* (27)
Immune system (human)	Neutrophils	$[Ca^{2+}]_i$ ↑, CD11b ↑	Howells *et al.* (252)
Immune system (rat)	Leukocytes, mast cells	$[Ca^{2+}]_i$ ↑, rolling ↑, adhesion ↑, migration ↑	Vergnolle (262) D'Andrea *et al.* (147)
Intestine (guinea pig)	Taenia coli	Relaxation (only AP)	Cocks *et al.* (376)
Intestine (guinea pig)	Submucosal neurons	Mast cell degranulation (tryptase) → hyperexcitability	Reed *et al.* (256)
Intestine (human)	Colon cancer cell lines (T84, Caco-2, HT-29, C1.19A)	Autocrine-secreted trypsinogen activates PAR_2	Ducroc *et al.* (446)
Intestine (human)	HT-29	$[Ca^{2+}]_i$ ↑, proliferation, TGF-α ↑, ERK-1/2 phosphorylation, EGFR transactivation	Darmoul *et al.* (447, 467)
Intestine (human)	Rectal mucosa biopsies	Cl^- secretion ↑, $[Ca^{2+}]_i$ ↑ required	Mall *et al.* (448)
Intestine (mouse)	Distal colon	Cl^- and K^+ secretion	Cuffe *et al.* (449)
Intestine (mouse)	Gastric fundus	Relaxation/contraction	Cocks *et al.* (376)
Intestine (mouse)	Model	Luminal proteinases → inflammation, PAR_2 ↑, granulocyte infiltration, wall thickness ↑, tissue damage ↑, T-helper cell type 1 cytokine	Cenac *et al.* (340)
Intestine (mouse)	Model	Modulate GI transit, L-type Ca^{2+} channel	Kawabata *et al.* (161)
Intestine (mouse)	Small intestine	Induce contraction through NK receptors (NK_1 and NK_2)	Zhao and Shea-Donohue (450)

TABLE 2. *Continued*

Tissue	Cell type	Effect	Refs.
Intestine (mouse)	Model (epithelial, neuronal)	TNBS-induced colitis ↓ (antiinflammatory), myeloperoxidase activity ↓, CD44 expression on lamina propria T lymphocytes ↓, IFN-γ ↓, IL-2 and IL-12 ↓, COX-2 ↓, iNOS ↓, TNF-α ↑	Fiorucci *et al.* (298)
Intestine (mouse)	Model	NO and capsaicin-sensitive afferent neurons involved in colonic inflammation and paracellular permeability	Cenac *et al.* (451)
Intestine (porcine)	Model	Opioid- and prostanoid-modulated anion secretion, neurogenic mechanism	Green *et al.* (313)
Intestine (rat)	Chief cells of gastric mucosa	Pepsin secretion ↑	Kawao *et al.* (452)
Intestine (rat)	Colon	Spinal Fos ↑, permeability ↑, (pot. visceral hyperalgesia)	Coelho *et al.* (308)
Intestine (rat)	hBRIE 380 (epithelial cell line)	$[Ca^{2+}]_i$ ↑, IP_3, AA, PGE_2, $PGF_{1\alpha}$	Böhm *et al.* (154) Kong *et al.* (253)
Intestine (rat)	Longitudinal (lm) and circular muscle (cim)	Biphasic contraction, relaxation; lm, contraction: L-type Ca^{2+} channel, sr-derived Ca^{2+}, PKC, PLC, TK; relaxation: L-type Ca^{2+} channel, TK, apamin-sensitive K^+ channels; cim: sr-derived Ca^{2+}, apamin-sensitive K^+ channels	Mule *et al.* (162, 163)
Kidney (mouse)	Cortical duct cells	Cl^- secretion	Bertog *et al.* (453)
Kidney (rat)	Afferent arteriole	NO dependent and independent vasodilation	Trottier *et al.* (347)
Nervous system (guinea pig)	Enteric neurons (intestinal)	Excitatory response PLC and $[Ca^{2+}]_i$ dependent	Gao *et al.* (319)
Nervous system (guinea pig)	Myenteric neurons	$[Ca^{2+}]_i$ ↑, PLC, sphingosine kinase	Corvera *et al.* (196) Garrido *et al.* (382)
Nervous system (CNS) (human)	A172 (gliablastoma cell line)	$[Ca^{2+}]_i$ ↑, PKC-α, PKC-γ, PKC-λ, proliferation ↓	Okamoto *et al.* (317)
Nervous system (wobbler mutant mouse)	Motor neuron	PAR_1 ↑ → motoneuron degeneration and neuronal cell death	Festoff *et al.* (193)
Nervous system (PNS) (rat and mouse)	Nociceptive primary afferent neurons	Fos ↑, SP ↑, NK_1 receptor activation, mechanical hyperalgesia, thermal hyperalgesia (via transactivation of capsaicin receptor)	Kawabata *et al.* (304) Vergnolle *et al.* (305) Kawao *et al.* (454)
Nervous system (PNS, sensory) (rat)	C-fibers	$[Ca^{2+}]_i$ ↑, SP ↑, CGRP ↑, edema ↑, plasma extravasation, leukocyte recruitment	Steinhoff *et al.* (243)
Nervous system (mast cell depleted rat)	Sensory neurons	Spinal Fos ↑	Kawabata *et al.* (307)
Nervous system (CNS) (rat)	Thoracic DRG	Enhances capsaicin- and KCl-evoked CGRP release, Fos ↑	Hoogerwerf *et al.* (314)
Pancreas (dog)	PDEC	$[Ca^{2+}]_i$ ↑, Cl^- and K^+ conductance ↑	Nguyen *et al.* (315)
Pancreas (human)	MIA PaCa-2	$[Ca^{2+}]_i$ ↑, PKC activation, inositol 1,4,5-triphosphate, proliferation	Kaufmann *et al.* (455)
Pancreas (mouse)	Pancreatic acini	Amylase secretion ↑, NO involved	Kawabata *et al.* (440)
Pancreas (mouse, rat)	Pancreatic duct cells	Pancreatic juice secretion	Kawabata *et al.* (311)
Pancreas (rat)	Pancreatic acini	Amylase secretion	Böhm *et al.* (154)
Skeletal muscle (rat)	Myoblasts	$[Ca^{2+}]_i$ ↑, proliferation	Chinni *et al.* (456)

TABLE 2. *Continued*

Tissue	Cell type	Effect	Refs.
Skin (human)	HDMEC	IL-6 ↑, IL-8 ↑, IL1β ↑, NFκB ↑, eNOS ↑	Shpacovitch *et al.* (263, 284)
Skin (oral mucosa) (human)	HSC-2, KB cells (oral epithelial cell line)	(RgpB)-induced: $[Ca^{2+}]_i$ ↑, IL-6 ↑; proteinase-3 activates PAR_2	Lourbakos *et al.* (28) Uehara *et al.* (341)
Skin (human)	Keratinocytes	$[Ca^{2+}]_i$ ↑, inhibits proliferation and differentiation, phagocytosis ↑, pigmentation, melanin ingestion ↑, IL-6 ↑, GM-CSF expression ↑, NFκB activation → ICAM-1 ↑, Rho-dependent phagocytosis	Santulli *et al.* (247) Derian *et al.* (275) Wakita *et al.* (283) Hou *et al.* (282) Schechter *et al.* (289) Steinhoff *et al.* (24) Seiberg *et al.* (457) Sharlow *et al.* (458) Buddenkotte *et al.* (287) Scott *et al.* (468)
Skin (human)	Mast cells	$[Ca^{2+}]_i$ ↑, degranulation; PAR_2 induces pruritus	D'Andrea *et al.* (147) Steinhoff *et al.* (24)
Skin (human)	NCTC2544 (keratinocyte cell line)	c-Jun activation, p38 phosphorylation, IKKα, IKKβ, NFκB activation (partially via PKC)	Kanke *et al.* (286)
Skin (mouse $PAR_2^{-/-}$)	Model	Type IV allergic dermatitis	Kawagoe *et al.* (288) Seeliger *et al.* (285)
Testes (human)	Fibroblasts, HEFF2, HMC-1	Tryptase and SLIGKV-induced proliferation via COX-2 → PGJ_2 → PPAR-γ	Frungieri *et al.* (245)
Testes (human)	Vascular cremaster muscle (perivenula injections)	Leukocyte rolling ↑, rolling velocity ↓, P-selectin ↑, inflammation	Lindner *et al.* (268)
Testes (rat)	Peritubular cells	$[Ca^{2+}]_i$ ↑, PKC activation	Meinhardt *et al.* (459)
Ureter (mouse)	Circular muscle	Beating ↑	Moffatt and Cocks (460)
Urogenital tract (human)	RT4	AA ↑, PGE_2 release, iPLA2 activation	McHowat *et al.* (170)
Cell line (Chinese hamster)	CHO	Ternary complex FVIIa/TF/FXa activates PAR_2	Riewald and Ruf (241)
Cell line (Chinese hamster)	CHO	Acrosin → $[Ca^{2+}]_i$ ↑	Smith *et al.* (26)
Cell line (Chinese hamster)	CHO	PAR_2 N-linked glycosylation regulates receptor expression and agonist sensitivity	Compton *et al.* (461)
Cell line (rat)	KNRK	$[Ca^{2+}]_i$ ↑, PKC mediates termination of signaling	Böhm *et al.* (154)
Cell line (human)	PC-3	Genistein → PAR_2 mRNA ↓	Li and Sarkar (462)
Cell line (human)	HMC-1	Induction of TNF-α secretion	Kim *et al.* (463)
Eye (human)	Corneal epithelial cells (HCECs)	$[Ca^{2+}]_i$ ↑, IL-6 ↑, IL-8 ↑, TNF-α ↑	Lang *et al.* (339)
Cell line (human)	LNCaP (left supraclavicular lymph node metastasis)	RhoA, cytoskeletal reorganization	Greenberg *et al.* (380)
	PAR_3-agonist mediated effects		
Blood (mouse)	Platelets	Platelet activation, PI hydrolysis, ATP secretion	Ishihara *et al.* (18, 324) Kahn *et al.* (19)
Blood ($PAR_3^{-/-}$, $PAR_4^{-/-}$ mouse)	Platelets	Protection against thrombosis, cofactor for thrombin cleavage	Nakanishi-Matsui *et al.* (39) Sambrano *et al.* (40) Weiss *et al.* (325)
Immune system (human)	Jurkat T-leukemic cells	Vav1, ZAP-70, SLP-76 phosphorylation	Bar-Shavit *et al.* (236)

TABLE 2. *Continued*

Tissue	Cell type	Effect	Refs.
		PAR_4-agonist mediated effects	
Airway (human)	HBEC (primary bronchial epithelial cells, A459, BEAS-2B)	IL-6 ↑, IL-8 ↑, PGE_2 ↑, $[Ca^{2+}]_i$ ↑	Asokananthan *et al.* (77)
Airway (mouse)	Tracheal SMC	Contraction/relaxation, PGE_2 ↑	Lan *et al.* (152, 153)
Airway (rat)	Epithelium, trachea, bronchi	Relaxation (epithelium-dependent)	Chow *et al.* (91)
Blood (human)	Platelets	Gingipain → $[Ca^{2+}]_i$ ↑, platelet aggregation; cathepsin G activates PAR_4	Sambrano *et al.* (330) Lourbakos *et al.* (329)
Blood (human)	Platelets	$[Ca^{2+}]_i$ ↑, ADP-dependent platelet aggregation, TXB_2 ↑	Andersen *et al.* (122) Chung *et al.* (334) Covic *et al.* (333) Kahn *et al.* (19) Henriksen and Hanks (117)
Blood (human, mouse)	Platelets	Activation, thrombosis protection	Sambrano *et al.* (40) Kahn *et al.* (19, 128)
Blood (human, mouse)	Platelets	Adenylyl cyclase inhibition via ADP release, P2Y12-receptor activation, G_i-dependent and -independent	Kim *et al.* (201)
Blood (rat)	Platelets	Endostatin release	Ma *et al.* (118)
Brain (rat)	Astrocytes	$[Ca^{2+}]_i$ ↑, toxic effect	Wang *et al.* (185)
Cardiovascular system (human)	Endothelium	IL-1α and TNF-α treatment → PAR_4 ↑ relaxation	Hamilton *et al.* (464)
GI tract (rat)	Esophageal smooth muscle	Relaxation	Kawabata *et al.* (371)
Immune system (rat)	Leukocytes	Rolling ↑, adhesion ↑, migration ↑	Vergnolle *et al.* (74)
Nervous system/intestine (guinea pig)	Enteric neurons	Excitatory response, dependent on PLC and $[Ca^{2+}]_i$ ↑	Gao *et al.* (319)
Vascular system (human)	VSMC	$[Ca^{2+}]_i$ ↑, ERK-1/2 activation, mitogenesis	Bretschneider *et al.* (220)
Transfected (*Xenopus*)	Oocyte	$[Ca^{2+}]_i$ ↑	Kahn *et al.* (19)
Cardiovascular system ($PAR_1^{-/-}$ mouse)	Cardiomyocytes, fibroblasts	Early ERK-1/2 activation involves G_q/PLC/PKC and Src; late ERK-1/2 activation involves Src, EGFR, and/or ErbB2; IP accumulation, hypertrophy	Sabri *et al.* (338)

↑, Up-regulated/activated; ↓, down-regulated; →, resulting in; AA, arachidonic acid; AMs, alveolar macrophages; ANF, atrial natriuretic factor; ASM, airway SMCs; BMCMC, bone marrow-derived cultured mast cells; BSMC, bronchial SMCs; CaMLCK, calmodulin myosin light-chain kinase; CASM, coronary artery SMCs; CHO, Chinese hamster ovary; CTGF, connective tissue growth factor; CVEGF, vascular endothelial growth factor C; Cyr61, cysteine rich protein 61; EDHF, endothelium-derived hyperpolarizing factor; eNOS, endothelial NO synthase; EPCR, endothelial protein C receptor; ErbB2, receptor protein-tyrosine kinase ErbB2 (erythroblastosis); FXa, factor Xa; Fyn, Src-family tyrosin kinase; GEC, glomerular epithelial cells; GEF, guanine nucleotide exchange factor; GMC, glomerular mesangial cells; Grb-2, growth factor binding protein-2; HAEC, human aortic endothelial cells; HAOAF, human adventitial fibroblasts; HASMC, human airway smooth muscle cells; HBE(C), human bronchial epithelial (cells); HCASMC, human coronary artery smooth muscle cells; HCEC, human corneal epithelial cells; HDMEC, human dermal microvascular endothelial cells; HFL, human fetal lung; HMC, human mast cells; HPAEC, human pulmonary artery endothelial cells; Hsp, heat-shock protein; HUR, human urothelial cells; LPS; lipopolysaccharide; MABP, mean arterial blood pressure; MEK-1, MAPK kinase-1; ncR-uPAR, noncoding RNA upstream of the *PAR1* gene; NHBE, normal human bronchial epithelial cells; NMDA, N-methyl-D-aspartate; NRVM, neonatal rat ventricular myocytes; P2Y12-receptor, platelet ADP receptor subtype; PBMC, peripheral blood mononuclear cells; PC, prostate cancer; PDEC, pancreatic duct epithelial cells; PI, phosphatidylinositol; PI_3P, PI-trisphosphate; PNS, peripheral nervous system; PPARγ, peroxisome proliferator-activated receptor γ; RANTES, regulated upon activation, normal T cells expressed and secreted; RASMC, rat aortic smooth muscle cells; RBCE, rat brain capillary endothelial cells; RgpB, arginine-specific gingipain B; ROCK, Rho-associated coiled-coil forming protein kinase; SAEC, small airway epithelial cells; SHP-1, SH2 domain containing nontransmembrane protein tyrosin phosphatase-1; TF, tissue factor; TFFs, trefoil factors; TK, tyrosine kinase; TNBS, trinitrobenzene sulfonic acid; TX, thromboxane; vWf, von Willebrand factor; WAF, wild-type p53 associated factor; WPB, Weibel-Palade body.

bin (*e.g.*, platelet aggregation, angiogenesis, endothelial cell permeability, vasoregulation, gene regulation, leukocyte trafficking, immunomodulation) could be attributed to the activation of its GPCR (40, 47–53).

PAR_1 has been detected in a variety of tissues, including platelets; endothelial cells; fibroblasts; monocytes; T cells positive for CD 8, CD 16, and either CD 56 or CD 57 (54, 55); natural killer (NK) cells (48); CD 34^+ hematopoietic progenitor cells (56); dental pulp cells (57); smooth muscle cells (SMC); epithelial cells; neurons; glial cells; mast cells; and certain tumor cell lines (17, 54, 58–61). A receptor with high affinity for thrombin has also been detected in rat peritoneal

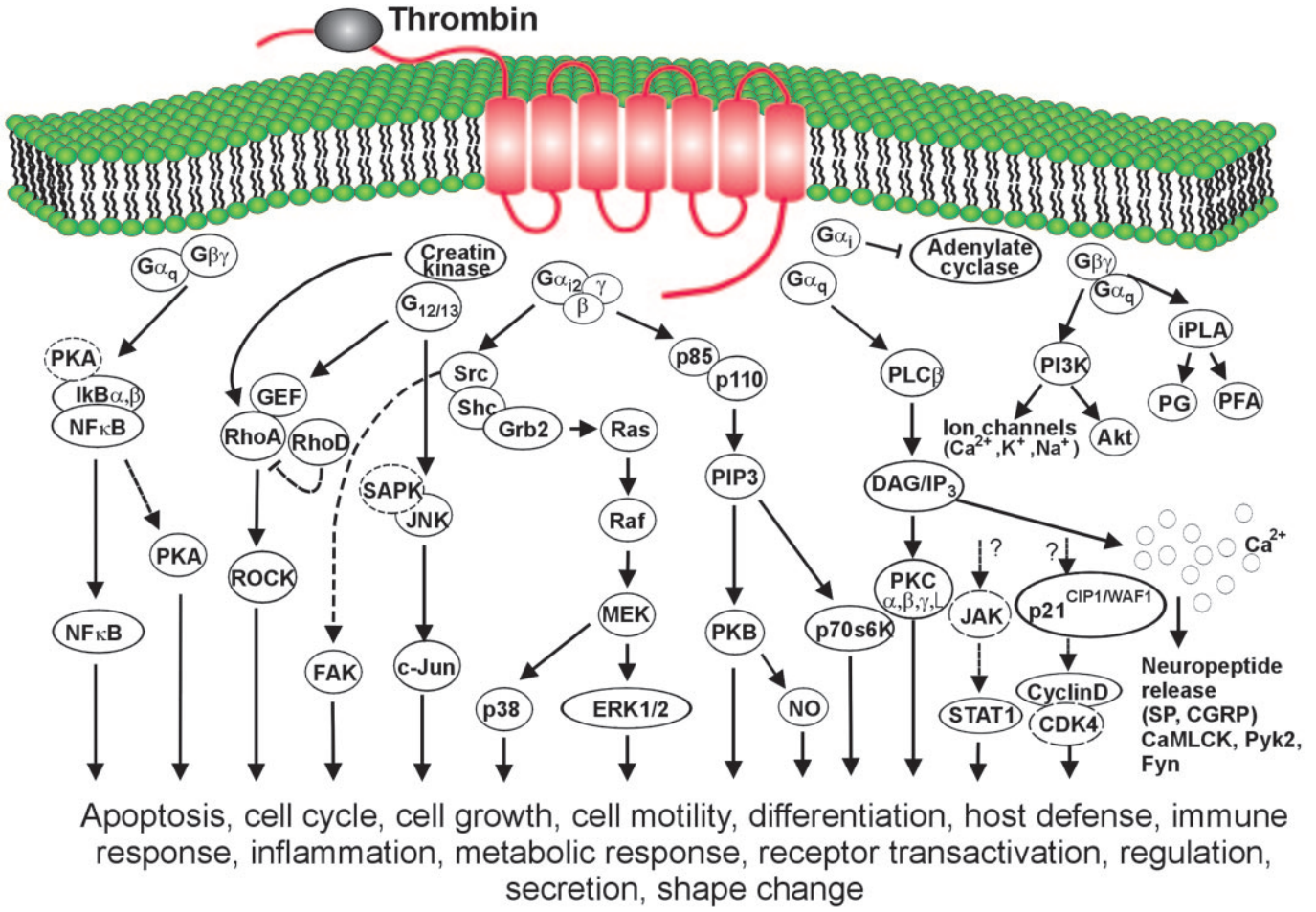

FIG. 2. Diagram showing the major G protein-mediated signaling pathways coupled to PAR_1. *Dashed lines* or *circles* represent signaling pathways or intermediates that are not fully revealed to be activated by PAR_1, but are in favor of other G protein-coupled receptors or are typical intermediate-accompanying molecules. Drawing represents a composition of signaling events of different tissues, cell types, and species (also see Table 2).

macrophages (62). It should be pointed out, however, that PAR_1 very likely does not represent the only target for thrombin, in that other (non-PAR) high-affinity binding sites for thrombin [*e.g.*, on platelets or macrophages (62)] and other conserved thrombin sequences apart from the catalytic domain (63) may also play a role in the cellular actions of thrombin in a variety of target tissues.

A. Vasculature and heart: PAR_1 antagonists—a novel potential approach for the treatment of cardiovascular diseases

PAR_1 can potentially regulate vascular function under both physiological as well as pathophysiological conditions (15, 42). A number of studies have revealed that thrombin and other agonists of PAR_1 can affect the vascular tone. For example, before the discovery of PAR_1, it was observed that thrombin can regulate vascular tone by an endothelial-dependent mechanism involving the release of nitric oxide (NO) (64). It is now recognized that this effect of thrombin is due to the activation of PAR_1. Moreover, thrombin and PAR_1 agonists can contract vascular SMC (VSMC) by a direct effect that requires extracellular Ca^{2+} (64). Thus, in isolated coronary artery and aorta preparations, PAR_1 mediates relaxation (64, 65). In contrast, PAR_1 agonists contract human placental and umbilical arteries (66). Furthermore, PAR_1 mediates prostanoid generation and secretion, cellular contraction and barrier dysfunction, and enhanced expression of platelet-derived growth factor (PDGF) in human and bovine endothelial cells (67). Interestingly, progestins such as progesterone or gestodene up-regulate PAR_1 expression and thereby stimulate thrombin-induced tissue factor-dependent surface procoagulant activity in the rat vascular system, suggesting a role of thrombin in hormone-induced thrombosis via PAR_1 activation (68).

Recent observations support a role of thrombin and PAR_1 in regulation of functions normal (69–71, 73) and atherosclerotic (75) endothelium. In normal human arteries, PAR_1 is mostly confined to the endothelium, whereas during atherogenesis, its expression is enhanced in regions of inflammation associated with macrophage influx, smooth muscle cell proliferation, and an increase in mesenchymal-like intimal cells (75). *In vivo*, a neutralizing antibody to PAR_1 has been observed to reduce expression of mRNA for the proliferating cell nuclear antigen, an index of intimal and

neointimal smooth muscle cell accumulation in rat arteries during balloon angioplasty. These data suggest that PAR_1 regulates proliferation and accumulation of neointimal SMC during tissue repair (76).

Thrombin and PAR_1 agonists cause a rapid but transient contraction of endothelial cells in various tissues resulting in gap formation and increased permeability of plasma proteins and inflammatory cells. Several mediators are involved in this PAR_1 modulated process such as cytokines, kinins, and biogenic amines (67, 72, 77–95).

It was also revealed that PAR_1 plays a crucial role during vascular ontogenesis. Interestingly, about 50% of the PAR_1-deficient mouse embryos die at midgestation with bleeding from multiple sites. However, a PAR_1 transgene driven by an endothelial-specific promoter prevented death of PAR_1-deficient embryos (96), indicating that PAR_1 modulates endothelial cell function in developing blood vessels, thereby contributing to vascular development and homeostasis in mice (88, 96).

Vascular wall cells respond to the procoagulant factor Xa by an increase in intracellular Ca^{2+} ($[Ca^{2+}]_i$) and by assembly of this factor into prothrombinase complexes that even enhance this effect. Additionally, factor Xa stimulation of PAR_1 leads to an increased production of tissue factor, a prothrombotic agent, underlining the important role of PAR_1 for thrombosis (86). Together, these results point to a pivotal role of PAR_1 in vascular homeostasis and thrombosis.

PAR_1 agonists are also mitogenic, stimulating proliferation of endothelial cells (97, 98), mediating endothelium-dependent relaxation to thrombin and trypsin in human pulmonary arteries (99), and causing the release of IL-6 from human microvascular endothelial cells (HMEC) (72). Because PAR_1 up-regulates α1(I)-procollagen synthesis in VSMC, one may speculate that PAR_1 plays a role in vascular wound healing (100).

Finally, factors that regulate PAR_1 function on endothelial cells have also been studied. For example, PAR_1 is down-regulated by shear stress (101) and inflammatory mediators such as TNF-α (102), and is directly modulated by thrombomodulin (103) in human endothelial cells. On the other hand, cyclic mechanical stress leads to an up-regulation of PAR_1 in VSMC (104). In contrast to PAR_2, little is known about the inflammatory mediators that regulate PAR_1 expression in unstimulated or stimulated endothelial cells.

As of yet, only a few studies exist about the role of GRKs in regulating PAR_1 in endothelial cells. Only recently, Tiruppathi *et al.* (105) found a crucial role of the isoform GRK-5 on thrombin-induced desensitization of PAR_1.

It is also necessary to mention the recently demonstrated role of PAR_1 in vascular associated pathological processes in which thrombin is involved. The role of PAR_1 in thrombus formation was recently investigated in an animal model. Cook *et al.* (106) examined the role of PAR_1 in intravascular thrombus formation in an experimental model of arterial thrombosis in the African green monkey. Using a blocking antibody to PAR_1, this group demonstrated a dramatically diminished thrombin-stimulated aggregation and secretion of human platelets, whereas platelet activation induced by the PAR_1 agonist SFLLR-NH_2 was not affected. These results demonstrated that a specific blockade of PAR_1 activation by thrombin can prevent arterial thrombosis in this animal model without significantly altering hemostatic parameters. The data suggest that PAR_1 is crucially involved in this disease and is an attractive antithrombotic therapeutic target, although it probably has to be inhibited in concert with the low-affinity thrombin receptor PAR_4 to fully prevent platelet activation (see *Section II.A*).

A demonstrated role of PAR_1 in intravascular thrombus formation and knowledge about side effects of thrombin inhibitors made appealing an idea to investigate the potency of PAR_1 antagonists as antithrombotic therapeutic drugs. Indirect thrombin inhibitors like heparin and warfarin, which prevent the circulation of thrombin, or the newly developed direct thrombin inhibitor ximelagatran (465) are currently used for the treatment of cardiovascular diseases, *e.g.*, myocardial infarction, atherosclerosis, and thrombosis. However, they carry potential bleeding liabilities as an undesirable side effect when administered in chronic patients. Because it is now well known that human platelet responses to thrombin are mainly mediated via PAR_1 (19), direct inhibition of this receptor instead of the proteinase represents an attractive way to circumvent possible side effects by thrombin inhibitors.

PAR_1 possesses several extra- and intracellular sites that are crucial for its function and that might represent possible targets for antagonists (107). On the extracellular side, blocking of the cleavage site or the hirudin-like domain could inhibit N-terminal cleavage. Bradykinin or peptides derived thereof, also known as thrombostatins, have been shown to prevent thrombin-induced platelet aggregation (108). In addition, antibodies directed against both the cleavage site and the hirudin-binding site have been generated (106, 109). Binding of the antagonist to either the tethered ligand or extracellular loop 2 prevents interaction of the novel N terminus with the extracellular loop. Several small synthetic antagonists have been developed that block the binding site of the tethered ligand. However, peptide antagonists based on modifications of the tethered ligand were relatively unstable or showed only limited inhibitory activity (35). Thus, a second generation of indole or indazole-based peptidemimetic antagonists was created that block binding of both the tethered ligand and the proteinase (110). One of these was recently shown to prevent thrombus formation in nonhuman primates (107). In addition, several nonpeptide inhibitors were developed. However, it was not reported whether these compounds were PAR_1-specific (111). On the intracellular side, the G protein binding sites are possible targets for pharmacological intervention. One approach used the transfection of endothelial cells with a Gα minigene (112). However, this might suppress all signaling pathways that involve Gα. Another more specific approach used so-called "pepducins," peptides that were derived from the third intracellular loop of PAR_1. These peptides were able to permeate the cells and also carried a membrane anchor (113, 114).

In summary, two preclinical studies with primates demonstrated that PAR_1 antagonists have indeed a therapeutic potential for the treatment of cardiovascular diseases (106, 107). However, extensive research needs to be done for the development of orally administered PAR_1 antagonists be-

cause only those drugs are thought to be suitable for chronic patients (111).

B. Platelets

Activation of platelets by thrombin or APs specific for PAR_1 is characterized by calcium influx; cytoskeletal reorganization; platelet aggregation; degranulation (17, 115, 116); thromboxane production (117); mobilization of the adhesion molecule P-selectin and the CD40 ligand to the platelet surface (119); stimulation of serotonin and epinephrine release (120); enhanced expression of CD62, PDGF (AB) and PDGF(BB) (121); and exposure of anionic phospholipids (phosphatidylserine, phosphatidylethanolamine) that support blood clotting (122). Thrombin has also been demonstrated to stimulate vascular endothelial growth factor release in megakaryocytes and platelets (123). The growth factor in turn is able to induce proliferation of endothelial cells. In addition, tissue inhibitor of matrix metalloproteinase (MMP)-4 that colocalized with MMP-2 in resting platelets was released upon platelet aggregation induced by collagen and thrombin. However, a direct effect using specific PAR_1 agonists has not been shown yet (124). The density of human PAR_1 on the platelet cell surface was estimated to be about 1500 molecules per resting platelet (125). A recently reported intronic polymorphism in the *PAR_1* gene leads to decreased expression on the platelet surface and thus to a lower response to the AP (126, 127). PAR_1 is a high-affinity thrombin receptor in humans: receptor blocking using an antagonist, domain specific antibodies, or simply by desensitization led to inhibition of responses at 1 nM thrombin but only to attenuated responses at 30 nM (128).

High-affinity thrombin binding of platelets is possibly associated via the membrane glycoprotein (GP)Ib-IX-V complex. This interaction is lost in patients suffering from the Bernard-Soulier syndrome, a disease where GPIb-IX-V is not expressed (129). The same condition can be mimicked using either monoclonal antibodies directed against GPIb or proteolytic removal of this protein from the platelet surface (130). Under these conditions, platelet aggregation is either delayed or requires higher thrombin concentrations.

Recently, Schmidt *et al*. (131) reported that the gene encoding IQ motif containing GTPase activating protein-2, a scaffolding protein for filopodal extension during platelet activation, was identified within the human *PAR* gene cluster at 5q13, flanked by the *PAR*1 gene and encompassing the *PAR*3 gene. Only thrombin- or PAR_1-AP-activated platelets showed a rapid translocation of IQ motif containing GTPase activating protein-2 to the platelet cytoskeleton. This suggests that a functional genomic unit evolved to mediate thrombin signaling events in humans (132).

PAR_1 on platelets also seems to play an important role during bacterial invasion: the cysteine proteinase gingipain from *Porphyromonas gingivalis* was reported to activate the receptor leading to uncontrolled activation of the host cells (28). The enzyme streptokinase derived from *Streptococcus sp*. is widely used in the treatment of coronary thrombosis. It functions as a plasminogen activator that forms an active complex with plasminogen of the host that is able to cleave PAR_1 (133). In mouse platelets, PAR_1 does not seem to play a role: PAR_1 expression was hardly detectable, and a specific AP did not activate rodent platelets (23, 134, 135). Moreover, platelets derived from PAR_1-deficient mice responded to thrombin-like wild-type platelets (135).

C. Immune cells

For some time, an important role for serine proteinases and PARs for the modulation of leukocyte effector functions has been proposed (136). As of yet, only limited data about the regulation of immune and inflammatory responses by PARs are available. It is well documented that PARs influence monocyte motility and chemotaxis, modulate pleiotropic cytokine responses, contribute to mononuclear cell proliferation, and induce apoptosis in various immune cells (84, 137). Recently, it was shown that PAR_1 is capable of stimulating elastase secretion from macrophages (138). Moreover, functional thrombin receptors are expressed on human T lymphoblastoid cells (139). However, the receptor subtype(s) (PAR_1, PAR_3, and PAR_4) have not been characterized as of yet. Human α-thrombin stimulated five different T lymphoblastoid cell lines to increase intracellular free Ca^{2+} concentrations and to activate protein kinase C (PKC), whereas thrombin receptors were absent in B cell lines. Thus, PARs may regulate T cell function during inflammation and immune responses, but the precise mechanism is still unknown. Granzyme A from cytotoxic and helper T lymphocytes appears to interact with PAR_1 in astrocytes to regulate thrombin function (140). Interestingly, granzyme A blocked the thrombin-induced platelet aggregation in a dose-dependent manner by cleaving PAR_1, presumably downstream from its thrombin targeted activation site, thereby reducing the response to subsequent challenge with thrombin; but granzyme A itself did not induce a signal in thrombin-stimulated platelets. Thus, granzyme A may interact with PAR_1 in a manner that is insufficient to cause aggregation, but sufficient to disarm the ability of the receptor to respond to thrombin. Finally, these observations demonstrate that granzyme A release occurring during immune responses within blood vessels would not directly cause platelet aggregation. Thus, the T cell-derived proteinase granzyme A seems to inhibit responses triggered by thrombin during inflammation or tissue injury.

PAR_1 modulates chemotaxis in inflammatory cells. Besides IL-8 secretion (71), thrombin induces production of monocyte chemoattractant protein-1 (MCP-1) in monocytes, probably via PAR_1 (58). However, other PAR_1-specific agonists were not used in this study. Therefore, it cannot be excluded that other thrombin receptors are involved. IL-8 secretion is up-regulated by interferon-γ (IFN-γ) and diminished by prostaglandin (PG)E_2 (51), suggesting a role in cytokine modulation. Furthermore, PAR_1 is capable of inducing IL-1 as well as IL-6 production in monocytes (82). These cytokines are known to be proangiogenic, implicating PAR_1 in angiogenesis and tissue repair. However, IFN-γ-differentiated growth-arrested U937 cells also respond to PAR_1 agonist administration by overcoming cell arrest and revert to a high proliferation rate via regulation of $p21^{CIP1/WAF1}$ and cyclin D_1. Together, these results may help to explain how

thrombin promotes tissue repair and unrestricted proliferation in malignant tissues (137, 141).

Because thrombin, via PAR_1, is chemotactic for large granular lymphocytes in humans (55), large granular lymphocytes can enhance the effects of PAR_1 in patients with inflammatory disorders (142–144). Moreover, thrombin modulates activity of NK cells (48). For example, thrombin can enhance NK cell-mediated cytotoxicity and IL-2 production in rheumatoid arthritis, and PAR_1 may therefore play an important role in this inflammatory process, as well as in NK cell responsiveness to IL-2.

D. Airways

Various cells in mammalian airways such as epithelial cells, SMC, and fibroblasts abundantly express PAR_1 on the cell surface. Several recent studies also suggest that PAR_1 may play an important role in inflammatory lung diseases such as neutrophilic alveolitis, pulmonary fibrosis, and asthma (50, 77, 91, 145–149).

In the airways of different species, PAR_1 exerts a dual role leading to stimulation of contraction on the one hand and relaxation on the other hand. For example, thrombin stimulates contraction of human bronchial rings (150) and constriction in guinea pig bronchi (as does AP) (151), but activates constriction as well as relaxation in mouse tracheal SMC (152, 153). In these mouse studies, trypsin, thrombin, and peptide agonists of PAR_1, PAR_2, and PAR_4 induced relaxant responses of isolated tracheal smooth muscle preparations, which were mediated by a prostanoid, probably PGE_2. This effect was abolished by indomethacin, the cyclooxygenase (COX)-2 inhibitor, nimesulide, and a prostanoid receptor antagonist (AH6809). PAR_1 and PAR_4 synthetic peptides induced a rapid, transient, contractile response that preceded the relaxant response. Interestingly, the relaxations but not the contractions were inhibited by indomethacin, indicating that this response is mediated by cyclooxygenase products.

E. Gastrointestinal tract

PAR_1 was detected in the lamina propria, the submucosa, endothelial cells, and nerves of the gastrointestinal (GI) tract (154–157). The GI tract expresses relatively high levels of PAR_1 mRNA compared with other tissues, both in mice and humans. The functional role of PAR_1 as a receptor mediating thrombin-induced effects in the GI tract is far from being resolved because thrombin can also interact with PAR_3 and PAR_4 (18, 20, 74). Several studies suggest a role for PAR_1 in regulating GI motility. In guinea pigs, PAR_1 mediates contraction of longitudinal smooth muscle tissue, dependent on extracellular Ca^{2+} (158, 159). In mouse intestine, PAR_1 agonists modulate the function of L-type Ca-channels and also cause contraction (161). Similar results have been observed in rats (162, 163). Irradiation by fractionated X-radiation leads to up-regulation of PAR_1 at the protein level, indicating a regulatory role of external trigger factors such as irradiation on PAR_1 expression (164). Stimulation of PAR_1 upon adhesion of pancreatic carcinoma cells to extracellular matrix proteins such as laminin, collagen IV, and fibronectin has been observed in a pancreatic carcinoma cell line (MIA PaCa-2) (165, 166). Other studies have pointed out a role for PAR_1 in intestinal secretory pathways. Buresi *et al.* (156) have shown that selective PAR_1 agonists stimulated Ca^{2+}-dependent chloride secretion in intestinal epithelial cells. This PAR_1-induced intestinal chloride secretion involves protein tyrosin kinase Src (Src), epidermal growth factor (EGF) receptor (EGFR) transactivation, activation of a MAPK pathway, phosphorylation of cytosolic phospholipase A_2 ($cPLA_2$), and cyclooxygenase activity. The presence of functional PAR_1 on intestinal epithelium and the fact that PAR_1 activation leads to ion secretion suggest that PAR_1 might have important implications for intestinal barrier functions. PAR_1 activation on intestinal surfaces could lead to a secretory response, thus contributing to diarrhea, a symptom of intestinal inflammation. More recent observations further suggest a role of PAR_1 in intestinal inflammation. We have shown that intracolonic administration of PAR_1 agonists caused inflammation and disruption of intestinal barrier integrity (53). Taken together, these results suggest a proinflammatory role of PAR_1 in the gut (reviewed in Ref. 167). However, additional studies using PAR_1 antagonists and/or PAR_1-deficient mice will have to verify the role of PAR_1 in the pathogenesis of inflammatory bowel diseases.

F. Kidneys and urogenital tract

Recently, a crucial role of PAR_1 in the cell-mediated renal inflammation of crescentic glomerulonephritis has been demonstrated *in vivo* (49). In wild-type mice treated with hirudin (a direct thrombin inhibitor, characterized by a bifunctional mechanism of inhibition, exclusive specificity and strong ability to bind the enzyme) and in PAR_1-deficient animals, the proinflammatory effect of thrombin was significantly reduced. Moreover, treatment of wild-type mice with the PAR_1 peptide agonist ($SFLLRN\text{-}NH_2$) augmented the inflammatory response, suggesting that PAR_1 plays an important role in renal inflammation *in vivo*. Unfortunately, as mentioned above, the peptide $SFLLRN\text{-}NH_2$ can also activate PAR_2 with a comparable potency, and therefore a role of PAR_2 in renal inflammation cannot be ruled out by this study. Surprisingly, although highly expressed in human kidneys, data clarifying a role for PAR_1 in inflammation of human kidneys are still lacking.

Recent data revealed that PAR_1 is involved in cellular invasion of a transfected canine kidney cell line. PAR_1 agonists abrogated $G\alpha_{(olf)}$-mediated invasion of MDCKts.src cells in collagen gels, indicating an important role for PAR_1 in tumor metastasis (168, 169). Using the human urogenital cell line RT4, studies done *in vitro* have revealed that PAR_1 and PAR_2 agonists are capable of activating $iPLA_2$ accompanied by release of PGE_2, which may provide cytoprotection during an acute inflammatory reaction (170).

G. Brain and peripheral nervous system

Recent studies are in favor of an important role of thrombin and PARs in the brain under normal and pathophysiological conditions such as trauma, inflammation, or tumorigenesis (171, 172). Under pathophysiological conditions, *i.e.*,

during breakdown of the blood-brain barrier, circulating thrombin in the bloodstream may enter the central nervous system (CNS), leading to activation of PAR_1 or PAR_4. Additionally, neurons and glia cells are capable of generating prothrombin (173). Most data so far have been achieved by investigating PAR_1 (13, 14, 174). In rat brain, PAR_1 is expressed by neurons of the neocortex, cingulate cortex, subsets of thalamic and hypothalamic nuclei, discrete layers of the hippocampus, cerebellum, and olfactory bulb, as well as by astroglia (60). Striggow *et al*. (175) showed that all four PAR subtypes are expressed in rat brain. PAR_1 expression was most abundant in the hippocampus, amygdala, and cortex. Interestingly, the expression of PAR_1, PAR_2, and PAR_3 was up-regulated during experimentally induced ischemia.

In human brain, PAR_1 is expressed in neurons and astrocytes (175). Cultured rat glia cells (C6) express both functional PAR_1 and PAR_2 (176, 177). Moreover, PAR_1 agonists induce up-regulation of inducible NO synthase (iNOS) in these cells (85) and promote neuronal survival after ischemia (178) or brain trauma (179). PAR_1 also protects astrocytes and neurons from apoptosis induced by hypoglycemia and oxidative stress during inflammation (180). In contrast, thrombin may also exert cytotoxic effects on neurons and induce neurite retraction (181, 182), which may be at least in part due to PAR_1. Functional studies further revealed that PAR_1 agonists cause retraction of neurites by neuroblastoma cells (181) and induce Ca^{2+} mobilization by hippocampal neurons (178).

In astrocytes, thrombin stimulates aggregation, morphological changes, and proliferation via PAR_1 and induces intracellular caspase pathways leading to apoptosis in a cultured motor neuron cell line (NSC19) (183, 184). Both PAR_1 and PAR_2 stimulate enhanced proliferation of astrocytes (185). Friedmann *et al*. (186) reported a key role of thrombin in PAR_1-mediated postinjury neuron survival. They demonstrated that the toxicity of thrombin on neurons can be controlled by down-regulation of PAR_1 and/or release of antithrombin III by T cells. Interestingly, prothrombin expression was enhanced 24 h after injury, whereas PAR_1, PAR_3, and nexin-1 mRNA expression was unchanged (187). Nexin-1 is a thrombin inhibitor that also appears to control thrombin-PAR_1 interactions in a rat trauma model (171, 187, 188). Thus, the whole factory needed to regulate and modulate thrombin function, including receptor as well as activating and inhibiting proteinases, can be generated in the brain. However, PAR_1 also induces the reversal of astrocyte stellation in mice and rat (189–191). A comparable process is caused by exogenous or endogenous injuries of the CNS, triggering astrogliosis.

Taken together, these data clearly indicate a functional role of PAR_1 during inflammation and injury in the CNS. However, in some studies using thrombin as an agonist, activation of other PARs and a role of other molecules like thrombomodulin cannot be excluded. For example, thrombin itself up-regulates thrombomodulin in astrocytes in a dose-dependent manner (192). Thus, future studies taking into account all CNS-derived PARs, their proteinases, and proteinase inhibitors are necessary to fully explore the role of PARs in the brain. Finally, some authors suggest a role of thrombin in Alzheimer's disease, amyotrophic lateral sclerosis, or HIV encephalitis, probably via activation of PARs (182, 193, 194).

Accumulating data also indicate the role of PAR_1 in the peripheral nervous system. In rat peripheral nerves, PAR_1 is expressed by primary afferent neurons (195). From these observations, one may speculate that thrombin may also regulate inflammatory responses in the peripheral nervous system via PAR_1. Indeed, very recently it has been demonstrated that PAR_1 is expressed by a large proportion of primary spinal afferent neurons (155). Thus, thrombin directly signals to sensory neurons by cleaving PAR_1. Moreover, administration of PAR_1 agonists can induce plasma extravasation and edema, which were blocked by ablation of sensory nerves and administration of antagonists to the neurokinin-1 receptor, supporting the idea that thrombin cleaves PAR_1 on sensory nerves to stimulate release of SP, which in turn interacts with the NK_1 receptor to induce neurogenic inflammation. Thus, thrombin triggers neurogenic inflammation via PAR_1 (155). However, because PAR_2 and PAR_4 mRNA are also expressed in the CNS, it cannot be excluded that proinflammatory effects of thrombin in the nervous system may, in addition, be mediated by other PARs. Functional studies further revealed that PAR_1 agonists induce Ca^{2+} mobilization in enteric neurons, and regulate both excitatory and inhibitory neurons of the myenteric plexus in guinea pig small intestine, *e.g*., by releasing neuropeptides (196).

Recently, Vergnolle and co-workers (197) as well as other groups (198) examined the effects of PAR_1 agonists on nociceptive responses to mechanical and thermal noxious stimuli. Interestingly, intraplantar injection of selective PAR_1 agonists induced an enhanced nociceptive threshold and withdrawal latency resulting in mechanical and thermal analgesia. However, thrombin was analgesic in response to mechanical, but not to thermal, stimuli. Moreover, application of PAR_1-AP with carrageenan significantly reduced the hyperalgesia. Thus, thrombin may play a dual nociceptive-analgesic role, and future studies will be required to determine whether PAR_1 agonists might be of therapeutic use for the treatment of pain.

H. Signaling by proteinases via PAR_1

Investigations during the last few years revealed that the above-mentioned PAR_1-mediated effects are transduced by various signaling pathways leading to diverse functions of PAR_1 under physiological and pathophysiological conditions. These findings were recently reviewed (13). However, in the current work we focus on the signaling events mediated via PAR_1 in different tissues and cell types (Table 2).

1. Platelets. After its generation from prothrombin, thrombin plays multiple roles in the blood coagulation cascade that are mediated by interaction with a number of physiological substrates, effectors, and inhibitors. The accumulation of thrombin at sites of vascular injury provides the recruitment of platelets into a growing hemostatic plug. When added to human platelets *in vitro*, thrombin causes platelets to change shape, stick to each other, and secrete the contents of their storage granules. How this is accomplished is still not fully

understood, but a major step forward occurred since the identification of PAR_1 in the 1990s. This finding shed light on the way by which an extracellular protease may initiate intracellular events.

Mammalian GTP-binding proteins (G proteins) fall into four families that are typically referred to by the designation of the α-subunit: G_s, G_i, $G_{q/11}$, and $G_{12/13}$. Human platelets express at least one member of the G_s family and four members of the G_i family (G_{i1}, G_{i2}, G_{i3}, and G_z), which, among other functions, stimulate or inhibit cAMP formation by adenylyl cyclase (199). In addition, platelets express one or more members of the $G_{q/11}$ family and members of the $G_{12/13}$ family (G_{12} and G_{13}) (199).

A number of different G protein α-subunits have been shown to bind to PAR_1, including members of the G_i, $G_{q/11}$ and $G_{12/13}$ families (200). However, the knowledge of these interactions in humans under physiological and pathophysiological conditions is still far from completion.

Kim *et al*. (201) have demonstrated that thrombin and PAR_1-AP, as well as PAR_4-AP, induce G_i pathway stimulation in human platelets. Additionally, the authors demonstrated that thrombin, PAR_1-AP, and PAR_4-AP cause platelet aggregation independently of G_i stimulation (201). Offermanns *et al*. (202) also demonstrated a direct interaction between G_{12}, G_{13}, and PAR_1. Additionally, it was reported that thrombin-mediated cleavage of the PAR_1 (and PAR_4; see *Section IV.B*) receptor leads to calcium-dependent and calcium-independent shape changes of human platelets in consequence of direct activation of both G_q and $G_{12/13}$ pathways, respectively (115, 202). Therefore, current evidence suggests that PAR_1 interacts with G_q, $G_{12/13}$, and possibly G_i protein family members in human platelets. In turn, these data suggest an activation of downstream signaling cascade members after PAR_1 stimulation on human platelets. Among such members are phospholipase C (PLC)-β, phosphoinositide 3 (PI3)-kinase, and monomeric G proteins.

Indeed, PI3 kinase was demonstrated to play an important role in some PAR_1 or thrombin-mediated cellular effects such as cytoskeletal reorganization, alterations in cell motility, cell survival, and mitogenesis. For example, thrombin is able to activate multiple PI3-kinase isoforms, including the recently discovered 110-kDa isoforms that can be directly activated by G protein $\beta\gamma$-subunits (145, 150, 203). Stimulation of platelets by PAR_1 leads to the activation of PI3 kinase, which is dependent on the small G protein Rho (204). Recently, Trumel *et al*. (205) have demonstrated that PI3 kinase plays an important role in the PAR_1-dependent reorganization of the platelet cytoskeleton via myosin heavy chain translocation and stable association of signaling complexes with the actin cytoskeleton. Interestingly, the activity of small G proteins such as Rac and cdc42 in platelets may be regulated through PAR_1 stimulation, but the role of PI3 kinase in these events remains to be determined (206). In their recent study, Vaidyula and Rao (207) provided evidence that in human platelets PLC-β2 plays a major role in responses to PAR_1 and PAR_4 activation, and that PLC-β2 is required for the sustained rise in $[Ca^{2+}]_i$ concentration upon thrombin activation.

Putting all of this together, current data suggest that thrombin activates human platelets by cleaving and activating PAR_1 and PAR_4. In turn, PAR_1 activates the members of $G_{q/11}$, $G_{12/13}$, and perhaps G_i families, leading to the activation of PI3 kinase, PLC-β, and monomeric G proteins (Rho, Rac, and possibly RapI), and also causes an increase of cytosolic Ca^{2+} concentration and inhibition of cAMP formation.

Additionally, it is important to note that despite the fact that human platelets express both PAR_1 and PAR_4, these receptors appear to be activated by different thrombin concentrations. Cleavage of human PAR_4 requires a higher concentration of thrombin than does cleavage of PAR_1, and it is likely that PAR_1 is the predominant signaling receptor at low thrombin concentrations (208, 209).

Mouse platelets provide an interesting contrast to human platelets: whereas human platelets express functional PAR_1 and PAR_4, mouse platelets express PAR_3 and PAR_4, although in the latter case signaling appears to be mediated entirely by PAR_4, with PAR_3 serving to facilitate PAR_4 cleavage at low thrombin concentrations (for details, see *Section IV.A*) (39, 40).

2. Cells in the nervous system. PAR_1, as mentioned above, appears to affect various processes in both the central and peripheral nervous systems (45, 210). Among such processes are: neuroinflammation and neurodegeneration, neuritogenesis, astrocyte proliferation, and synaptic plasticity. However, here we would like to focus on data concerning the involvement of PAR_1 in intracellular signaling cascades in neuronal cells.

PAR_1 may have an effect on various intracellular signaling cascades within neuronal cells (171, 181, 189). By coupling to different G proteins, PAR_1 affects a wide range of neuronal cell functions. For example, the necessity of PAR_1 coupling to G_{12} was demonstrated for thrombin-stimulated DNA synthesis in 1321N1 astrocytoma cells (211). Interestingly, injection of antibodies directed against G_{12} abolished the thrombin-stimulated DNA synthesis (212). Additional studies performed on 1321N1 astrocytoma cells revealed that thrombin treatment causes a concentration-dependent rounding. One may speculate that such an effect of thrombin could be Rho-dependent. The Rho family of small GTPases is known to be involved in the control of cytoskeletal changes via modulation of actin polymerization. Indeed, this thrombin-induced rounding of 1321N1 astrocytoma cells was Rho-dependent and mediated via G_{12} (213).

Moreover, LaMorte *et al*. (214) demonstrated binding of PAR_1 to G_q by using the same cell line. The authors also showed that thrombin stimulation induces Ras-GTP complex formation and that Ras is required for PAR_1-mediated activation of PLC in 1321N1 astrocytoma cells (214). This is especially interesting because thrombin is known to be a factor regulating the proliferation of astrocytes. This effect of thrombin appears to be associated with $G_{o/i}$- and G_q-mediated pathways. Wang and coworkers recently shed some light on the downstream cascade events of PAR_1 signaling (174, 210). G_q-Mediated PLC activity results in Ca^{2+} mobilization and activation of PKC, which phosphorylates a nonreceptor tyrosine kinase, proline-rich tyrosine kinase (Pyk2). Interestingly, the activation of other nonreceptor tyrosine

kinases like Src and focal adhesion kinase (FAK) was also demonstrated after thrombin stimulation. Pyk2 is a factor that is able to connect GPCRs to ERK1/2 activation. Therefore, Pyk2, acting together with Src-tyrosine kinase, causes the activation of the ERK/MAPK pathway, which mediates the proliferative effect of thrombin. Additionally, the same group of authors demonstrated that the $G_{o/i}$-mediated PI3 kinase pathway is also involved in thrombin-induced astrocyte proliferation (174).

Thrombin is also known to exhibit both beneficial and unfavorable effects in hippocampal neurons and astrocytes (178, 180, 215). Donovan and colleagues (184, 216) found that tyrosine kinases, serine/threonine kinases, and the actin cytoskeleton are involved in both pro- and antiapoptotic effects of thrombin in neuronal cells. However, there was no involvement of $G_{o/i}$ and the PI3 kinase pathway observed in these thrombin effects.

Additionally, Zieger *et al.* (217) demonstrated the existence of a novel PAR_1-associated signaling pathway in the nervous system. According to these data, PAR_1 participates in cAMP-independent PKA activation in SNB-19 glioblastoma cells. Moreover, PAR_1 stimulation causes the activation of transcriptional factor nuclear factor κB (NFκB) in this cell type (217).

In summary, besides morphological and proliferative effects described above that are mediated via $G_{12/13}$, G_q, and $G_{o/i}$, respectively, thrombin also affects activation of transcriptional factors such as activator protein-1 (AP-1) in neuronal cells (214).

3. SMC. Thrombin exerts direct effects on vascular cells such as SMC and endothelial cells via interactions with PARs (218). As mentioned in *Sections II.H.1* and *II.H.2*, PAR_1 interacts with $G_{12/13}$, G_q, and G_i to elicit diverse downstream signaling events. For example, thrombin-stimulated DNA synthesis and cell migration are associated with activation of the G_{13} signaling pathway in SMC. The G_{13} signaling cascade includes the activation of Rho and thus induction of cytoskeletal changes affecting cell migration (219). The G_q-dependent signaling pathway includes the activation of PLC, which in turn leads to MAPK phosphorylation and receptor tyrosine kinase transactivation, both necessary events in thrombin-mediated proliferation. It is interesting that the thrombin-mediated activation of MAPKs such as ERK1/2 was recently demonstrated in SMC. As noticed, PAR_1 caused rapid phosphorylation, whereas PAR_4 induced prolonged phosphorylation of these kinases in SMC (220). Additionally, Ghosh *et al.* (221) have shown that thrombin activates p38 MAPK in a time-dependent manner in VSMC. Furthermore, Sabri *et al.* (222) demonstrated that PAR_1 agonists induce activation of Jun N-terminal kinase (JNK) and Akt/PKB in rat ventricular cardiomyocytes.

After dissociation of the G protein heterodimer, $G\beta\gamma$ interactions activate phosphoinositide 3-kinase, which promotes $[Ca^{2+}]_i$ release that is required for SMC growth in response to thrombin stimulation (223).

It is also interesting that PAR_1 signaling can modulate gene transcription induced by cytokines in SMC. Thrombin, acting via PAR_1, can block IL-6-induced signal transducer and activator of transcription 3/Sis-inducible factor-A (Stat3/SIF-A) activation (224).

In summary, since the discovery of PARs, considerable progress in our understanding of thrombin signaling in SMC was achieved. This allowed some light to be shed on signaling events associated with SMC proliferation, migration, and synthesis of extracellular matrix proteins (*e.g.*, collagen) after thrombin stimulation. However, additional studies are necessary to reveal the role of thrombin in SMC apoptosis, vascular lesion formation, and wound-healing associated pathways.

4. Endothelial cells. Thrombin is known to affect various functions of endothelial cells. Among these are cell rounding, changes of cell-cell junctions, proliferation, barrier function, and permeability. These thrombin-induced effects appear to be mediated via PARs, particularly PAR_1 (225, 226). A major step forward was reached in recent studies, which revealed important intracellular signaling events underlying PAR_1-mediated effects in endothelial cells (227).

PAR_1 was demonstrated to interact with $G_{o/i}$, G_q, and probably $G_{12/13}$ in endothelial cells (228, 229). As well as it was demonstrated for neuronal cells, PAR_1-mediated cytoskeletal changes in endothelial cells (cell rounding) are associated with the activation of RhoA (225). Additionally, in the same work it was demonstrated that PAR_1-AP stimulation rapidly enhanced vascular permeability in a mouse skin assay (225). Moreover, it was found that binding of PAR_1 to pertussis toxin (PTX)-sensitive G proteins (however only to G_o, but not to G_i) is also necessary for thrombin-induced changes of endothelial barrier permeability (229). The activation of the MAPK signaling pathway by thrombin in endothelial cells was also demonstrated. This activation plays a crucial role in thrombin-induced effects of endothelial cell functions such as chemokine and cytokine production as well as the expression of cell adhesion molecules (89, 230, 231).

The role of thrombin and PAR_1 in the activation of transcriptional factor networks was intensively investigated in recent publications. Malik and coauthors (228, 231, 232) studied the involvement of PAR_1 in the activation of NFκB in endothelial cells. Their studies showed that $G_{\alpha q}$ and the $G_\beta\gamma$ dimer are responsible for NFκB activation and intercellular adhesion molecule-1 (ICAM-1) transcription in endothelial cells induced by the PAR_1 agonist thrombin and the PAR_1-specific AP TFLLRNPNDK. Furthermore, transfection experiments strongly supported simultaneous activation of $G_\beta\gamma$/PKC-δ/p38 and $G_\beta\gamma$/PI3-kinase pathways that converge into the Akt pathway, leading to subsequent NFκB activation and ICAM-1 expression (228, 231, 232). Moreover, thrombin-induced stimulation of vascular cell adhesion molecule-1 (VCAM-1) production involves the inducible binding of p65 NFκB to a tandem NFκB motif in the 5′ flanking region (233). Taken together, these findings suggest that in the case of ICAM-1 and VCAM-1, p65 NFκB is necessary for transducing the thrombin response in endothelial cells.

Additionally, thrombin-mediated induction of VCAM-1 was shown to involve the inducible binding of GATA-2 to a tandem GATA motif in the upstream promoter region (234). Interestingly, the effect of thrombin on GATA-2 DNA bind-

ing and transcriptional activity was found to be mediated by a PI3K, PKC-ς-dependent signaling pathway (233).

It is important to note that thrombin effects on transcriptional factor networks have been more deeply investigated in endothelial cells than in other cell types. These data explain, at least in part, thrombin-mediated effects on the production of cell adhesion molecules and some chemokines in endothelial cells. Subsequently, this accounts for thrombin effects at leukocyte migration via endothelial barrier (ICAM-1 serves as a ligand for leukocyte β_2 integrins and promotes leukocyte adhesion) and leukocyte recruitment.

5. Immune cells. The participation of PARs in T cell signaling pathways has also been demonstrated (235). Recently, Bar-Shavit *et al.* (236) have examined a possible involvement of Vav 1 in PAR-mediated signaling in human Jurkat T cells. The Vav family has three known members in mammalian cells (Vav, Vav2, and Vav3) and one in nematodes (CelVav) (237). Tyrosine phosphorylation of Vav1 regulates its activity as a guanine-nucleotide exchange factor for the Rho-like small GTPases RhoA, Rac1, and cdc 42, which affect cytoskeletal reorganization and activation of stress-activated protein kinases/JNKs. Bar-Shavit *et al.* (236) clearly showed that activation of PARs induces tyrosine phosphorylation of Vav1 in Jurkat T-leukemic cells. Because ζ-chain-associated protein kinase of 70 kDa (ZAP-70) and SH2-domain containing leukocyte-specific phosphoprotein of 76 kDa (SLP-76) have been shown to associate physically with Vav1 and because this association is critical for normal functioning of T cells, it was tempting to investigate whether ZAP-70 and SLP-76 are involved in PAR-induced signaling cascades. Indeed, an increase of tyrosine phosphorylation of ZAP-70 and SLP-76 was observed after activation of Jurkat cells with PAR-APs. Moreover, phosphorylation of Vav1 in response to PAR stimulation was dependent on $p56^{lck}$ (236). Unfortunately, because nonselective PAR-APs were used in the studies done with the Jurkat cells (236), it is difficult to distinguish between the effects of PAR_1 and PAR_2 in this T cell model system. Furthermore, because of the lack of the ability of PAR_3-derived peptides to activate PAR_3 (39), the role of PAR_3 in T cell signaling remains unknown.

6. Factor Xa signaling mediated via PAR_1. Most of the PAR_1-associated signaling events have been observed subsequent to cell stimulation by thrombin. However, recently a possible role of PAR_1 in coagulation factor Xa-mediated signaling has been demonstrated (238–240). The coagulation proteinase factor Xa is generated at sites of vascular injury and inflammation after formation of the tissue factor/VIIa (TF/VIIa) complex. Coagulation factor Xa has been shown to be mitogenic for SMC (238) and elicits inflammatory responses in endothelial cells. Riewald and Ruf (241) have presented new data indicating the involvement of PAR_1 in Xa-mediated signaling. They used HeLa cells expressing only PAR_1 and demonstrated that factor Xa induces NFκB activation and MAPK phosphorylation in these cells. Inhibition studies with specific antibodies revealed that factor Xa responses were mediated via PAR_1 (241). Thus, factor Xa (or potentially other serine proteinases) may substitute for thrombin in proteolytic signaling via PAR activation. In endothelial cells, which also express PAR_2, Camerer *et al.* (240) have shown that factor Xa signaling was mediated not only by PAR_1, but also to a large extent via PAR_2. Together, PAR-1 and PAR-2 appear to account for more than 90% of factor Xa signaling in endothelial cells (240).

In summary, the multiple biological as well as inflammatory and immune responses of thrombin that are mediated by PAR_1 [including 1) vasoregulation; 2) increased vascular permeability; 3) cellular adhesion and infiltration of leukocytes; 4) angiogenesis; 5) stimulation of the production of inflammatory mediators such as cytokines, neuropeptides, NO and prostanoids, for example; 6) regulation of extracellular matrix proteins; and 7) induction of signal transduction pathways which are involved in immunomodulation] suggest an important role of PAR_1 during inflammation and immune response.

III. PAR_2 in Inflammation and Immune Response

PAR_2 is expressed in brain, lia (DRG), eye, airway, heart, GI tract, pancreas, kidney, liver, prostate, ovary, testes, and skin (21–22, 24, 154, 242–245) and is found in various cell types such as epithelial cells, endothelial cells, SMC, osteoblasts, as well as immune cells such as T cells, neutrophils, mast cells, or eosinophils (97, 154, 246–254). On the other hand, platelets do not express PAR_2 (248). Recent findings point to an important role for PAR_2 under physiological and pathophysiological conditions in many tissues (Table 2). However, the endogenous enzymes responsible for activating PAR_2 in many tissues remain to be determined. Many endogenous or exogenous trypsin-like enzymes may cleave and activate PAR_2. Expression of trypsinogen-2 mRNA and its translation product has been demonstrated in endothelial cells (255). Interestingly, various types of human cancer cells secrete enzymes with trypsin-like specificity (255) that may activate PAR_2. In human skin, trypsinogen-4 generated by keratinocytes and trypsinogen-2 from human dermal microvascular endothelial cells can activate PAR_2 *in vitro* (M. Steinhoff, unpublished observation). Another candidate is mast cell tryptase, a major secretory protein of human mast cells. Mast cells from mice and rats are more heterogeneous regarding their protease content, although they also produce proteases with tryptic specificity. Tryptase has been shown to activate PAR_2 on epithelial as well as endothelial cells and neurons (23, 196, 243, 251, 256). The observation that tryptase can activate PAR_2 suggests a role of this receptor in humans under circumstances when mast cells are involved, *e.g.*, during inflammation, hypersensitivity reactions, and wound repair (24, 257). However, the ability of human tryptase to activate PAR_2 appears to be restricted by receptor glycosylation at an N-terminal residue just proximal to the receptor's cleavage activation site (258, 259). Notwithstanding, the effects of tryptase on cells *in vitro* often mimic those of PAR_2 activation: tryptase up-regulates IL-1β and IL-8 secretion, enhances the presence of intracellular adhesion molecules/selectins on endothelial cells, mediates accumulation of neutrophils and eosinophils, produces vascular leakage, and is mitogenic for epithelial cells, fibroblasts, and SMC (260–263). That said, direct proof that PAR_2 mediates the effects of tryptase during inflammation *in vivo* is still lacking.

A. Vasculature

In the vasculature, PAR_2 has many effects that are proinflammatory. Agonists of PAR_2 induce relaxation in the rings of rat aorta or porcine coronary artery dependent on endothelial NO synthase activity (246, 248, 250). This effect is abolished in the absence of endothelium (246, 250). In contrast, trypsin stimulates contraction of the rabbit aorta in the absence of endothelium (264). In the intact rat, iv injection of SLIGRL-NH_2 produces a marked fall in blood pressure, consistent with release of NO from endothelial cells (248). Furthermore, PAR_2 agonists increase IL-6 production (72), induce von Willebrand factor release, and serve as a mitogen for human umbilical vein endothelial cells (HUVEC) (97, 265, 266).

Moreover, it was demonstrated that some inflammatory mediators are able to affect the expression of PAR_2 in endothelial cells. In HUVEC, PAR_2 mRNA is up-regulated by TNF-α and IL-1α, cytokines that act together to orchestrate the acute inflammatory response (25, 265).

In summary, PAR_2 mediates vasodilation, plasma protein extravasation, as well as endothelial cell proliferation in the cardiovascular system. Thus, this receptor can be regarded as an important factor in neovascularization. Moreover, PAR_2 can be considered as a vascular sensor for trypsin-like proteinases of the coagulation cascade, which play an important role in cardiovascular medicine.

B. Immune cells

Although information has been acquired about the role of PAR_2 in epithelial and endothelial function, relatively little is known so far about the role of this receptor in the immune system. As was recently demonstrated, PAR_2 is expressed by various cells involved in immune response, such as T cell lines, eosinophils, neutrophils, and mast cells (147, 160, 249, 252, 267). Accumulating evidence points to a role of PAR_2 in the regulation of leukocyte function. Some of the PAR_2-mediated effects on leukocyte behavior have been observed *in vivo* in rodents (262). In this model, it has been reported that the PAR_2-AP (SLIGRL-NH_2) caused a significant increase in leukocyte migration into the peritoneal cavity after ip injection. Furthermore, PAR_2-APs induced a significant increase in leukocyte rolling and adherence by a mechanism depending on the release of platelet-activating factor (262). Lindner *et al*. (268) have also demonstrated the importance of PAR_2 for leukocyte adhesion and rolling. In a model of acute inflammation induced by tissue trauma, they showed that PAR_2-deficient mice have a decreased leukocyte rolling capacity compared with wild-type mice. Conversely, activation of PAR_2 by a specific PAR_2-AP in wild-type mice induced a significant reduction of leukocyte rolling velocity, an increased leukocyte rolling flux as well as increased leukocyte adhesion (268).

Howells *et al*. (252) reported that a specific PAR_2-AP as well as trypsin are able to activate PAR_2 on human neutrophils. PAR_2 activation caused shape changes and up-regulation of CD11b/CD18 in these cells. In a coculture of human neutrophils with endothelial cells, PAR_2 agonists induced L-selectin shedding and CD11b/CD18 up-regulation in neutrophils (268). We demonstrated that PAR_2 agonists induce $[Ca^{2+}]_i$ release in human neutrophils and enhance neutrophil motility in 3-D collagen gel lattices (269). Moreover, Lourbakos *et al*. (27) demonstrated that a bacterial proteinase, gingipain-R from *P. gingivalis*, activates PAR_2 on human neutrophils. Because many bacteria with pathogenicity in humans produce serine proteinases with trypsin-like activity, it can be assumed that certain pathogenic effects of bacteria in many tissues can be mediated through PAR_2. For example, Lourbakos *et al*. (28) reported that activation of PAR_2 by bacterial gingipain R leads to IL-6 secretion in human oral epithelial cells. In mouse airways, PAR_2 agonists were capable of inhibiting the recruitment of neutrophils induced by bacterial proteinase (270), suggesting a direct role of PAR_2 on neutrophil function *in vivo*. From these data, one may speculate that PAR_2 may serve as a receptor for serine proteinases derived from bacteria, viruses, or even fungi and may thus directly trigger inflammatory or immune responses *in vivo* induced by microorganisms. However, this intriguing hypothesis remains to be verified.

Recent work has shed some light on the role of PAR_2 on eosinophil function. Miike *et al*. (267) clearly demonstrated that human eosinophils express functional PAR_2. Furthermore, trypsin as well as a specific PAR_2-AP was able to induce degranulation and superoxide production in human eosinophils. More recently, Temkin *et al*. (79) reported that human mast cell tryptase induced up-regulation of IL-8 mRNA and caused IL-8 release from human eosinophils. A comparable effect of tryptase on eosinophil IL-6 expression and release was also observed. This effect of tryptase appears to be activated via the protein kinase/AP-1 signaling pathway (79). Schmidlin *et al*. (271) have also demonstrated that PAR_2 mediates infiltration of eosinophils and hyperreactivity in allergic inflammation of the airway. Furthermore, PAR_2 activation releases survival factors such as granulocyte-macrophage colony-stimulating factor (GM-CSF) from eosinophils (272). Together, these results are clearly in favor of an important role of PAR_2 in eosinophil regulation and in inflammatory/allergic diseases in which eosinophils are involved.

Several T cell lines also express PAR_2 (244). Trypsin as well as a synthetic PAR_2-AP induce $[Ca^{2+}]_i$ mobilization in the Jurkat and HPB.ALL T cell lines (249). In Jurkat cells, the involvement of PARs, possibly PAR_2, in Vav1-mediated signaling was clearly demonstrated (236).

The localization of PAR_1 and PAR_2 on human mast cells was first reported by D'Andrea *et al*. (147). As shown by immunohistochemistry, PAR_2 was localized not only on the cell membrane of mast cells, but also on the membrane of the intracellular tryptase-positive granules (147). Moreover, rat peritoneal mast cells also express PAR_2 mRNA (273). However, the precise role of PAR_2 in mast cells especially with regard to a potential autocrine regulatory mechanism via tryptase activation requires additional investigation. In summary, these results provide evidence for a role of serine proteinases in directly regulating immune cells and immune responses via PARs. An understanding of the molecular mechanisms by which PAR_2 is involved in immune regulation will aid the design of new antiinflammatory drugs that can target proteinase-activated receptors.

C. Arthritis

Arthritis is a chronic inflammatory disease that is characterized by joint swelling, vasodilatation, edema, hyperemia, pain, and recruitment of inflammatory cells, especially neutrophils and macrophages, but also lymphocytes. Very recently, it was demonstrated that PAR_2 participates in the pathophysiology of arthritis (274). Using PAR_2-deficient mice, the authors showed that PAR_2 deficiency results in a marked reduction of swelling responses of experimentally induced monoarthritis. However, no differences were observed concerning joint damage when compared with wild-type mice. Using an enzymatic approach (β-galactosidase), they also showed positive β-gal staining in endothelial cells of blood vessels. Two weeks after induction of monoarthritis, however, a significant increase of β-gal staining of extravascular inflammatory cells was observed in $PAR_2^{+/+}$ mice compared with $PAR_2^{-/-}$ mice. Examination of the knee joint showed dramatic arthritic changes in $PAR_2^{+/+}$ mice characterized by synovial hyperplasia, an enhanced inflammatory infiltrate, and cartilage damage. Thirty days after the induction of monoarthritis, the cartilage was completely replaced by pannus in $PAR_2^{+/+}$ mice. In contrast, $PAR_2^{-/-}$ mice showed an intact appearance of the cartilage similar to normal and control joints. This is in favor of an important role of PAR_2 as a mediator of proinflammatory responses in the joints. Moreover, a novel synthetic PAR_2 peptide agonist (ASKH95, phenylacetyl-LIGKV-OH) revealed proinflammatory effects after intraarticular injection like synovial hyperemia and joint swelling. These results clearly demonstrate that ASKH95 induces signs of chronic inflammation such as long-lasting swelling responses, vasodilatation, and tissue destruction. Interestingly, ASKH95 showed a longer-lasting proinflammatory effect in the tissue compared with the classical SLIGRL PAR_2 agonist peptide. This may be due to different rates of degradation, longer receptor activation by ASKH95, or most likely different lipophilicity. The underlying mechanisms of the PAR_2-mediated inflammatory effect in this chronic disease, however, are not fully explored yet. Potential mediators may be cytokines or prostanoids, for example. Thus, PAR_2 may play an essential role in the pathophysiology of arthritis, and PAR_2 antagonists may be beneficial for the treatment of this chronic inflammatory disease.

D. Skin

PAR_2 may play an important role in cutaneous inflammation. Keratinocytes and dermal endothelial cells express functional PAR_2 and are important targets of inflammatory mediators (247, 275, 276). In humans, PAR_2 is also expressed by dermal immunocompetent cells, which so far have not been characterized (24), and by dermal sensory nerves (277). Potential endogenous activators of PAR_2 in human skin are mast cell tryptase, proteinases of the trypsin-family produced by keratinocytes (trypsinogen-4), and proteinases of the fibrinolysis cascade, such as factor VII/Xa, which could be released during inflammation and wound healing. Exogenous activators of PAR_2 may be serine proteinases generated by bacteria, fungi, and house dust mites, although direct evidence on keratinocytes is still lacking. Indirect evidence for bacterial-induced activation of PAR_2 is coming from the observation that bacterial gingipain activates the receptor on buccal keratinocytes (28). At certain stages of atopic dermatitis or psoriasis, mast cells are in close contact to basal keratinocytes or are recruited into the epidermal layer (278). Numerous mast cells are found in the dermis in close proximity to blood vessels and at the dermal-epidermal border in atopic dermatitis and psoriasis. Thus, tryptase or other skin-derived proteinases may activate PAR_2 to induce inflammatory changes and thereby contribute to the pathophysiology of atopic dermatitis and psoriasis. It is well known that intradermal injection of tryptase into the skin results in vasodilatation and erythema, followed by leukocyte infiltration and local induration (279), indicating a role of tryptase in cutaneous inflammation. Moreover, tryptase is an important long-term marker in body fluids for systemic anaphylaxis and other immediate hypersensitivity allergic reactions (280). Thus, it is possible that PAR_2 mediates the systemic effects of tryptase in certain skin diseases.

Serine proteinases other than tryptase may also activate PAR_2. Cytotoxic T cells, which express PAR_2 (249), also produce the trypsin-like enzyme granzyme A (281), suggesting a potential role for PAR_2 in regulating T cells in inflammatory dermatoses. In summary, proteinases in the skin are signaling molecules that may directly regulate the function of target cells by activating PAR_2. PAR_2 as well as PAR_1 agonists regulate cytokine production such as IL-8 (282), IL-6, and GM-CSF (283) in cultured human keratinocytes. Furthermore, PAR_2 may also regulate proliferation and differentiation in the skin. Similar to TGF-β, PAR_2 agonists inhibit proliferation or differentiation of human neonatal keratinocytes, whereas PAR_1 agonists stimulate proliferation (275). In contrast, agonists of both PAR_2 and PAR_1 are mitogenic for endothelial cells (248). Thus, our observations in normal and inflamed human skin suggest a functional role of PAR_2 in different cell types of human skin during inflammation.

In human skin, PAR_2 is only weakly expressed by microvascular endothelial cells. Studies done *in vitro* revealed that PAR_2 is functionally expressed by human dermal microvascular endothelial cells and that PAR_2 agonists induce up-regulation and release of IL-6 and IL-8 in a concentration-dependent fashion (263). Moreover, PAR_2 regulates expression of cell adhesion molecules in primary cultures of human endothelial cells (284) and cell lines (268). These findings are supported by *in vivo* findings demonstrating a role of PAR_2 in the adhesion, rolling, and extravasation of leukocytes in rat venules. Finally, PAR_2 appears to play a role in the regulation of leukocyte/endothelial interactions in humans and mice *in vivo*, as shown for atopic dermatitis or experimentally induced contact dermatitis (285). Together, these results strongly support the idea that PAR_2 plays an important role in leukocyte/endothelial interactions during inflammation and immune response.

The knowledge about the signaling cascades involved in PAR_2-induced inflammatory and immune responses by keratinocytes is still incomplete. Recently, Kanke *et al.* (286) have shown that PAR_2 agonists stimulate activation of inhibitory κB kinases (IKK)α and IKKβ, and also stimulate NFκB-DNA binding. Our own data using primary human keratinocytes also clearly demonstrate that PAR_2 activates NFκB (287).

Moreover, PAR_2 is directly involved in the NFκB-mediated regulation of keratinocyte ICAM-1, which is an important molecule for the regulation of keratinocyte immune responses such as host defense, allergy, and recruitment of inflammatory cells into the epidermis (287).

Finally, recent data suggest a role of PAR_2 during cutaneous inflammation *in vivo*. In a murine model of experimentally induced allergic contact dermatitis, a proinflammatory role of PAR_2 agonists was demonstrated (285, 288). Stimulation of PAR_2 resulted in increased ear swelling responses with a maximum after 48 h. Independently, our group confirmed the proinflammatory effects of PAR_2 agonists in a model of experimentally induced allergic and irritant contact dermatitis using PAR_2-deficient mice. Moreover, we examined the underlying mechanisms responsible for PAR_2-induced effects during contact dermatitis (285). These results clearly indicate that PAR_2 is involved in edema formation, plasma extravasation, up-regulation of cytokines (IL-6), cell adhesion molecules (ICAM-1), and selectins (E-selectin) in mice. Intravital microscopy studies further showed that velocity and adhesion of leukocytes is impaired in PAR_2-deficient mice compared with controls. Moreover, *in vivo* studies by microdialysis confirmed a role of PAR_2 in mediating vascular responses such as edema formation and plasma extravasation also in human skin. Finally, *ex vivo* studies in skin biopsies of human volunteers are in favor of a role for PAR_2 in selectin regulation of dermal blood vessels because PAR_2 agonists induced a marked increase of E-selectin immunoreactivity in dermal endothelial cells in comparison to control tissues. Together, these results support a regulatory role of PAR_2 during cutaneous inflammation and immune response.

E. Airways

Several observations are in favor of an important role of PAR_2 in airway inflammation. Firstly, tryptase (251, 289) seems to play an important role in airway inflammation and hyperresponsiveness (253). Secondly, PAR_2 is markedly up-regulated after exposure to proinflammatory stimuli or cytokines (265) that have been shown to play a role in chronic airway diseases. Morphological studies have demonstrated PAR_2 immunoreactivity in endothelium and smooth muscle of bronchial vessels. These observations are consistent with a similar distribution in other tissues and with the known ability of PAR_2 agonists to cause arterial relaxation as well as contraction in certain arteries (290). Moreover, PAR_2 is expressed by bronchial, tracheal, epithelial and SMC which may result in direct or indirect bronchomotor effects leading to pathophysiological conditions in the airways such as asthma or chronic obstructive pulmonary disease. Interestingly, PAR_2 agonists are capable of causing bronchoconstriction (291). Whether this response is dependent on secondary effects such as the generation of bronchoactive peptides or other mediators remains to be determined. However, studies done *in vitro* suggest further that PAR_2 agonists also produce a relaxant effect in isolated main bronchi (292). Thus, PAR_2 agonists may cause dose-dependent bronchoconstrictor or bronchodilatator effects in the airways, with high agonist concentrations favoring bronchoconstriction. NO and prostanoids like PGE_2 may be involved in this process, because potentiation of PAR_2-induced bronchoconstriction has been observed upon inhibiting NO synthase or after prostanoid generation and COX-2 activation (153). In conclusion, there is strong evidence that PAR_2 stimulation activates contractile and dilator mechanisms in the airways. The reason why the predominant effect in guinea pig airways *in vivo* is bronchoconstriction, however, is not known at present. In addition to the potentially bronchoprotective effects observed by Cocks *et al*. (292) in rat bronchial preparations, others have shown that PAR_2 may also mediate bronchoprotection in guinea pig airways (293). However, other observations show that PAR_2 can act as an enhancer of histamine-mediated contraction (294). The study of Cocks *et al*. (292) showed that trypsin colocalized with PAR_2 in airway epithelial cells, where PAR_2 activation resulted in relaxation of airway preparations by the release of cytoprotective PGE_2. Prostanoids may be important mediators of PAR_2 activity in the airways not only in the lung, but also in the GI tract (152, 153, 253, 295). It is tempting to speculate that tryptase and/or other proteinases with trypsin-like activity, released in close proximity to the cells expressing PAR_2, may stimulate this receptor during airway inflammation. Finally, intensive staining of PAR_2 in the apical region of the airway epithelium might suggest an activating role of proteinases, for instance bacterial proteinases, present in the airway lumen (296). Very recently, PAR_2 was shown to stimulate the release of MMP-9 in a human epithelial cell line (297), providing a strong hint that PAR_2 is involved in the orchestration and reorganization of lung extracellular matrix proteins.

It was also revealed that PAR_2 stimulation of airway epithelial cells leads to the release of eosinophil survival-promoting factors such as GM-CSF (272). Moreover, in human respiratory epithelial cells, PAR_2, like PAR_1, stimulates IL-6, IL-8, and PGE_2 release (77).

Profound evidence for an involvement of PAR_2 in allergic inflammation of the airways is provided by the recent work of Schmidlin *et al*. (271). To study the involvement of PAR_2 in airway inflammation, they used PAR_2-deficient mice and mice overexpressing human PAR_2 (PAR_2tg). PAR_2 is known to be up-regulated by inflammatory agents. Sensitization of wild-type mice by injection of ovalbumin led to infiltration of immune cells into the lumen of the airways and induced hyperreactivity, whereas both infiltration of eosinophils into the lumen and airway hyperreactivity was exacerbated in PAR_2tg mice. In contrast, infiltration of immune cells and airway hyperreactivity was markedly diminished in PAR_2-deficient mice. Additionally, in $PAR_2^{-/-}$ mice the IgE response was strongly reduced, implicating a role of PAR_2 in immune response. Remarkably, intranasal administration of PAR_2-AP in wild-type mice stimulated increased recruitment of macrophages, confirming the proinflammatory role of PAR_2 in the airways, whereas altered PAR_2 expression mainly influenced eosinophil infiltration.

In summary, evidence exists for a proinflammatory as well as antiinflammatory role of PAR_2 in airway inflammation. This dual role may be explained by the various tissues, cells, methodological approaches, and inflammatory model systems (acute *vs.* chronic, mouse strains) that have been used to evaluate this role of PAR_2 in the airways. So far, it appears

that PAR_2 is a sensor receptor that releases proinflammatory mediators in the early phase and antiinflammatory molecules in the late phase of "regulated inflammation." Under "dysregulated" inflammatory situations such as chronic disease states, PAR_2 may exert antiinflammatory effects, as shown in a colitis model (298), or proinflammatory effects, as demonstrated in an asthma model (271). Thus, additional studies using human and mouse (knockout) models of pulmonary dysfunction are necessary to fully explain the role of PAR_2 during inflammation and immune response.

F. Brain and peripheral nervous system

By immunohistochemistry, PAR_2 has been localized in various compartments of the nervous system such as brain, spinal cord, DRG, and peripheral nerves, with different receptor densities found during various stages of mouse development (299). At d 14, PAR_2-staining was observed throughout the mouse brain. Among the hippocampal formation, PAR_2 was localized in the subiculum, in pyramidal cells throughout the CA1, CA2, CA3, and hilus region, and in granular cells of the dentate area. Additionally, staining for PAR_2 was observed in all cortical layers, amygdaloid nuclei, striatum, thalamus, and hypothalamus (175). Peripheral nerves were intensively stained after d 14. Similarly, PAR_2 has also been detected in human brain (276). In rat brain, PAR_2 is also expressed by neurons of the hippocampus (175, 300), meningeal cells (301), as well as astrocytes (176, 185). PAR_2 is abundantly expressed in various subareas and is transiently up-regulated during oxygen and glucose deprivation (175). Moreover, PAR_2 activation is cytotoxic for isolated rat hippocampal neurons in a concentration-dependent manner (300).

Domotor *et al.* (302) recently reported that agonists of PAR_2 induce Ca^{2+} responses in brain microvascular endothelial cells. This effect may be modulated by elastase and plasmin, which regulate PAR_2 signaling. Studies of the actions of PAR_2 agonists on CNS targets suggest an important role of PAR_2 during injury, growth, apoptosis, and probably memory.

Very recently, PAR_2 has been localized on rat sensory neurons (243). Moreover, functional data strongly support the idea that the peripheral nervous system is directly regulated by PAR_2 during neurogenic inflammation. Neuropeptides such as calcitonin gene-related peptide and substance P (SP) from primary spinal afferent neurons are known as important mediators of neurogenic inflammation in many organs. Because of the close proximity of tryptase-containing mast cells to spinal afferent fibers, and because agonists of PAR_2 cause effects similar to those of tryptase in many tissues, comprising many of the characteristics of neurogenic inflammation, it was speculated that serine proteinases or other PAR_2 agonists may activate PAR_2 on sensory neurons to stimulate neuropeptide release and may thus regulate inflammation. Indeed, it was shown that agonists of PAR_2 can induce inflammation by a neurogenic mechanism that depends on the release of calcitonin-gene related peptide (CGRP) and SP from primary spinal afferent neurons. However, there also appears to be a nonneurogenic component of PAR_2-induced inflammation because granulocyte infiltration triggered by PAR_2 in a paw edema inflammation model was unaffected by sensory denervation or by neuropeptide receptor antagonists. Thus, PAR_2 agonists may also act directly on endothelial cells or on neutrophils themselves to stimulate inflammation-related granulocyte adhesion and infiltration (14, 250). It is possible that serine proteinases may activate PAR_2 on spinal afferent neurons under pathophysiological inflammatory circumstances. In a similar manner, serine proteinases may regulate enteric neuronal function by cleaving PAR_2 in a Ca^{2+}-dependent manner (196). Trypsin, which is released by airway epithelial cells, but not the PAR_2 agonist peptide sequence SLIGRL, induced a marked contraction in guinea pig bronchi via SP release. Thus, non-PAR mediated effects of trypsin may also regulate neuropeptide function in the lung (303).

Several reports strongly suggest a role of PAR_2 in the central transmission of pain and nociception in rats and mice (304–306). Intraplantar administration of low doses of PAR_2 agonists, which do not induce inflammation, resulted in a long-lasting hyperalgesia. Interestingly, these effects of PAR_1 agonists were more efficacious in comparison with other inducers of hyperalgesia such as PGE_2 (0.3 μg). In the spinal cord, PAR_2 agonists induced an up-regulation of c-fos, a marker of activated nociceptive neurons (305, 307, 308). Another study also reported a role of PAR_2 in a rat visceral pain model (308). PAR_2 agonists administered *in vivo* clearly increased abdominal colonic contractions. This effect was inhibited by an NK_1 receptor antagonist, but not by the PG inhibitor indomethacin (308). However, additional studies are necessary to fully explain the underlying direct or indirect effects of PAR_2-induced nociception. For example, neuropeptides released from neurons upon PAR_2 stimulation may activate the release of nociceptive mast cell mediators such as kinins or prostanoids (309).

The observation that PAR_2 agonists are involved in the transmission of neurogenic inflammation and pain subsequently draws the question whether PAR_2 may be involved in the central transmission of pruritus. Itching is one of the most frequent symptoms in dermatological diseases and accompanies inflammatory and immune responses of many diseases such as hypersensitivity reactions or urticaria, for example. Indeed, neuronal PAR_2 appears to be involved in the induction of pruritus in human skin (277). Moreover, the endogenous PAR_2 agonist tryptase was increased up to 4-fold in atopic dermatitis patients, and PAR_2 expression was markedly enhanced on primary afferent nerve fibers in skin biopsies of atopic dermatitis patients. Intracutaneous injection of specific PAR_2 agonists provoked enhanced and prolonged itch when applied intralesionally. Thus, PAR_2 activation on cutaneous sensory nerves may be a novel pathway for the transmission of itch and inflammatory responses during atopic dermatitis and probably other skin diseases. PAR_2 antagonists may be promising therapeutic targets for the treatment of cutaneous neurogenic inflammation and pruritus (277).

In summary, PAR_2 may play an important regulatory role in the central and peripheral nervous system in normal and disease states, including neurogenic inflammation.

G. Digestive tract and pancreas

In rats, Kawabata *et al*. (167, 310, 311) detected PAR_2 mRNA in the sublingual, submaxillary, and parotid salivary glands. Of these three distinct salivary glands, the sublingual exhibited the strongest responses to PAR_2 activation resulting in the secretion of mucin. This response appeared to be mediated in part via a tyrosine kinase signal pathway, because genistein, an inhibitor of several tyrosine and other protein kinases, attenuated the effect. Pretreatment with the alkaloid capsaicin, which activates the transient receptor potential of vanilloid type 1 and which, in turn, is known to play a major role in inflammatory thermal nociception, failed to abrogate the secretion of mucin from salivary glands but abolished the PAR_2-triggered cytoprotective secretion of mucus in the stomach. These results provide both a sensory neuron-independent and -dependent mechanism for PAR_2-regulated exocrine secretion (312). Additional support for a role of PAR_2 in salivary gland secretion was provided by data showing that a PAR_2 agonist can stimulate amylase secretion from rat parotid gland slices *in vitro* (311).

In the GI tract, PAR_2 is highly expressed by enterocytes, where it is localized on the apical and basolateral membranes (253, 313). Moreover, myocytes of the muscularis mucosae and muscularis externa as well as neuronal elements are immunoreactive for PAR_2. Recent observations indicate that trypsin may regulate enterocytes by cleaving and triggering PAR_2 at the apical membrane (253) to induce the generation of PGE_2 and PGF_{1a}, suggesting that PAR_2 may have a cytoprotective as well as an inflammatory effect on the GI tract. Moreover, mucosal mast cell proteinases may possibly activate PAR_2 on enterocytes and colonic myocytes. PAR_2-mediated inhibition of intestinal motility may contribute to inflammatory conditions in which mast cells are involved.

Trypsin may activate PAR_2 in the pancreas itself under physiological and pathophysiological conditions because trypsin can be prematurely activated in the inflamed pancreas (314). PAR_2 seems to play an important role also in pancreatic nociception because injection of an AP specific for PAR_2 can activate and sensitize pancreas-specific afferent neurons *in vivo* (314). Moreover, Hoogerwerf *et al*. (314) found that stimulating DRG with a PAR_2-AP resulted in enhanced capsaicin- and KCl-stimulated release of calcitonin gene-related peptide, which is a marker for nociceptive signaling.

PAR_2 is expressed by both acinar cells, which release digestive enzymes, and duct cells, which produce fluid and bicarbonate. In isolated pancreatic acini, trypsin and PAR_2 agonists stimulate amylase release (154) (N. W. Bunnett, personal communication). *In vivo* studies revealed the secretion of pancreatic juice after PAR_2 activation (167). Interestingly, PAR_2 agonists applied to the basolateral but not apical membrane of monolayers of pancreatic duct cells increase short circuit currents due to activation of Ca^{2+}-sensitive Cl^- and K^+ channels (315). These effects may be of relevance in pancreatitis when trypsin is released across the basolateral membrane.

H. Signaling by proteinases via PAR_2

There are relatively few studies examining the involvement of PAR_2 in signaling cascades compared with the relatively large number of studies concerning PAR_1-mediated intracellular signaling (13, 14). So far, there are only indirect data indicating that PAR_2 interacts with G_q/G_{11} (activation of calcium signaling) and possibly with G_0/G_i (316). Whether or not PAR_2 binds to other G proteins, such as G_{12} or G_{13}, remains unclear.

Interaction of PAR_2 with G_i and G_q suggests a subsequent activation of PLC, PKC, and MAPK pathways. These signaling pathways can affect various cell activities including cell proliferation, morphological changes, motility and survival, and gene transcription regulation (Table 2 and Fig. 3). Indeed, as demonstrated for neuronal cells and SMC, stimulation by tryptase as well as PAR_2-AP leads to subsequent activation of PLC and PKC (317, 318). Kanke *et al*. (286) demonstrated that PAR_2 agonists (trypsin as well as the AP SLIGKV-NH_2) stimulate JNK and p38 MAPK activation in a human keratinocyte cell line (NCTC2544). Moreover, Vouret-Craviari *et al*. (225) described PAR_2-induced activation of RhoA in HUVEC revealing a possible mechanism underlying PAR_2-mediated effects at cytoskeleton in macrovascular endothelial cells. Together, these data shed some light on the potential signaling mechanisms underlying PAR_2-associated cellular effects such as depolarization response in neuronal cells, proliferation and mitogenic response in SMC, proliferation and differentiation of keratinocytes, and cytoskeletal changes in endothelial cells (225, 275, 319, 320).

Additionally, the effects of trypsin as well as PAR_2-AP on the activation of nuclear transcriptional factors have recently been demonstrated. It was shown that PAR_2 agonists stimulate NFκB-DNA binding activity and activation of upstream kinases IKKα and IKKβ (286). The effects of PAR_2 stimulation on NFκB or IKK also have been described in other studies performed on different cell types (13, 263, 287, 297, 321).

It is also important to pay attention to factor Xa signaling mediated via PAR_2. It was mentioned in *Section II.H.6* that this factor induces signaling events via PAR_1; the potential role of PAR_2 in such signaling remained unclear for a long time. Recently, however, the involvement of PAR_2 activation in coagulation factor Xa signaling has been reported. In HUVEC, factor Xa interacts with a protein called effector cell proteinase receptor-1 (EPR-1). This interaction is associated with signal transduction, generation of intracellular second messengers, and modulation of cytokine gene expression. Inhibitors of factor Xa blocked these responses (322). Additionally, direct cleavage of PAR_2 by factor Xa has been demonstrated, reflecting the complexity of PAR_2-induced signaling. These data allowed the authors to suggest that factor Xa induces endothelial cell activation via a novel cascade of receptor activation involving docking to EPR-1 and proteolytic cleavage of PAR_2 (322).

As already mentioned extensively in *Section II.H*, PAR_1 and PAR_2 both account for around 90% of endothelial factor Xa-mediated signaling in mice. In contrast, PAR_1 virtually accounts for all factor Xa-induced activation in fibroblasts, indicating potential cell-specific synergistic effects of PAR receptors on target cells (25).

Moreover, Koo *et al*. (323) demonstrated an involvement of PAR_2 in factor Xa signaling by analyzing factor Xa-induced

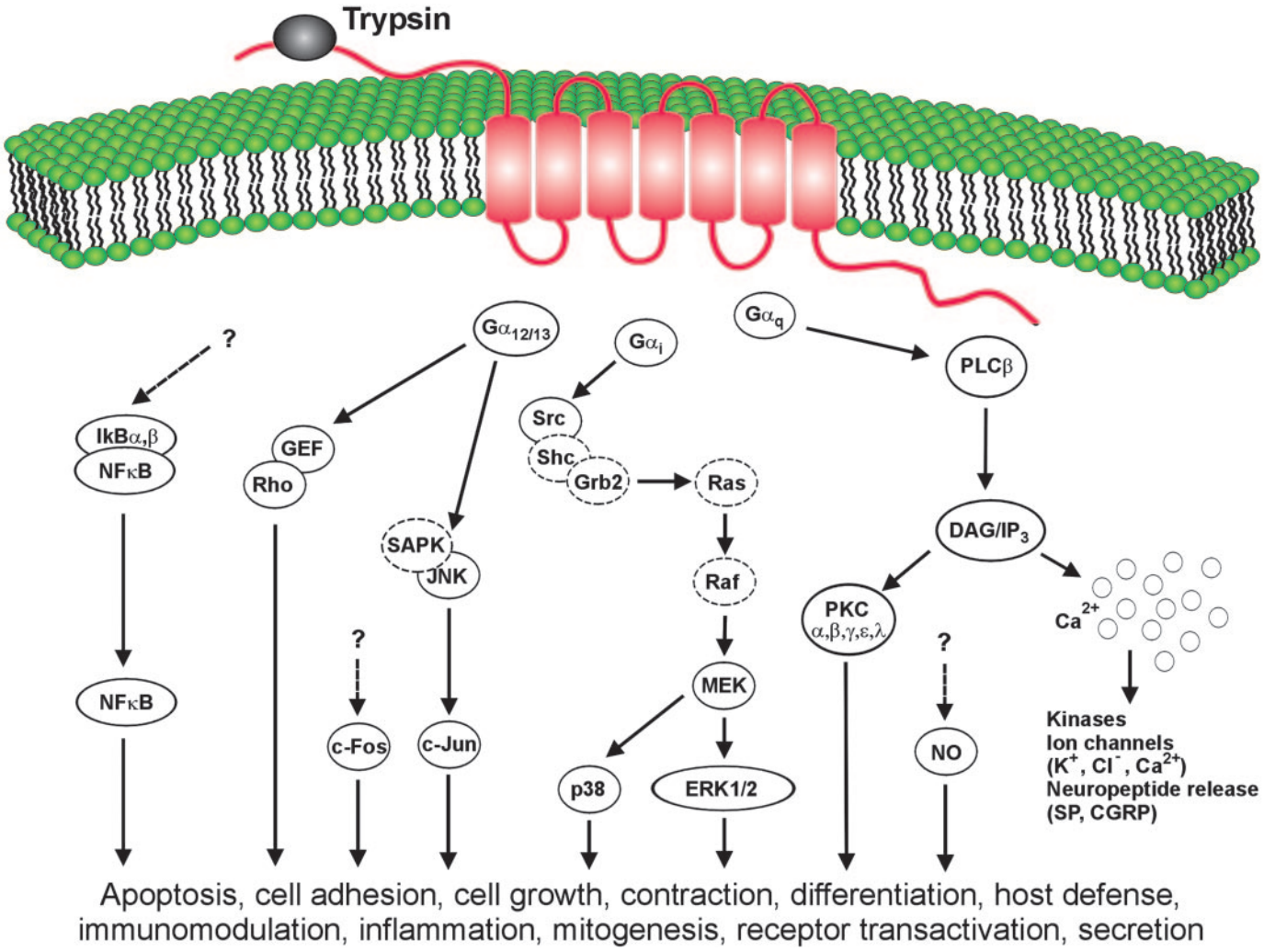

FIG. 3. Diagram showing the major G protein-mediated signaling pathways coupled to PAR_2. *Dashed lines* or *circles* represent signaling pathways or intermediates that are not fully revealed to be activated by PAR_2, but are in favor of other G protein-coupled receptors or are typical intermediate-accompanying molecules. Drawing represents a composition of signaling events of different tissues, cell types, and species (also see Table 2).

stimulation of coronary artery SMC using cell proliferation and ERK1/2 activation as indices of response. Furthermore, factor Xa-induced ERK1/2 activation was not desensitized by preincubation of the cells with thrombin. However, ERK1/2 activation was markedly attenuated by prior exposure of the cells to PAR_2-AP (SLIGKV-NH_2). The mitogenic effect of factor Xa was significantly reduced in the presence of an anti-PAR_2 monoclonal antibody that attenuates receptor activation, demonstrating the specificity of these effects (323). Together, these observations suggest that various signaling cascades are involved in PAR_2-mediated signaling. Clearly, we are just beginning to understand the variety of PAR_2-induced cell signaling pathways regulated under physiological conditions and during disease.

IV. PAR_3 and PAR_4

A. Biology and distribution of PAR_3 and PAR_4

As already mentioned, murine platelets do not express PAR_1. The observation that thrombin was still capable of inducing Ca responses in these cells led to the identification of PAR_3 (18). In humans, PAR_3 is expressed in bone marrow, heart, brain, placenta, liver, pancreas, thymus, small intestine, stomach, lymph nodes, and trachea, although the cell types remain to be identified. In mouse, PAR_3 is expressed by megakaryocytes and platelets, among other cell types. The receptor is necessary for normal thrombin signaling in mouse platelets because blocking of the hirudin-like domain of PAR_3 using a specific antibody prevented mouse platelet activation by low but not by high concentrations of thrombin. The same result was observed with PAR_3-deficient platelets (19, 324). This also shows that PAR_3 is a high-affinity thrombin receptor in mouse that is activated by proteolytic cleavage. Interestingly, murine PAR_3 ($mPAR_3$) itself does not lead to thrombin signaling even when overexpressed, indicating that the receptor has lost its ability to function autonomously during evolution (39, 40). Knocking out the *PAR3* gene in mice leads to protection of the animals against thrombosis but has a relatively mild effect on hemostasis (325) (Table 2). However, analysis of PAR_3 expression in human platelets

showed that the receptor is not produced or is hardly produced by these cells (128). This suggests that in humans PAR_3 does not play a major role for platelet activation in contrast to the mouse system.

Antibodies specific for PAR_1 inhibited human platelet activation by low but not by high concentrations of thrombin (125, 326). In mice, PAR_3 is necessary for normal thrombin signaling in platelets. This indicates the presence of more than one thrombin receptor on the surface of these cells. Indeed, a fourth receptor was cloned, named PAR_4 (19, 20, 128). So far, PAR_4 has been cloned from human, mouse, and rat tissues (19, 20, 327). In humans, PAR_4 is widely expressed in brain (175), testes, placenta, lung, liver, pancreas, thyroid, skeletal muscle, and small intestine (20). In rats, PAR_4 is expressed in esophagus, stomach, duodenum, jejunum, distal colon, spleen, and brain (327, 328).

Both in mice and humans, platelets utilize two thrombin receptors. PAR_4 is a low-affinity receptor in platelets both in humans and mice. However, mouse platelets use PAR_3 and PAR_4 instead of PAR_1 and PAR_4 to respond to thrombin (19). In humans, platelet effects of thrombin appear to be mediated predominantly by PAR_1. Only at high concentrations and when PAR_1 activation has been inhibited is platelet activation by thrombin dependent on PAR_4. Signals mediated by PAR_4 result in calcium influx (19, 122, 329), thromboxane production (117), endostatin secretion in rat platelets (118), and platelet aggregation (122) (Table 2). However, selective activation of human PAR_4 ($hPAR_4$) resulted in a weaker response compared with $hPAR_1$-mediated signaling because anionic phospholipids were not exposed on the surface of $hPAR_4$-activated platelets. Interestingly, $hPAR_4$ can also be activated by cathepsin G, a neutrophil granule proteinase (330). Cathepsin G mediates neutrophil-platelet interactions at sites of vascular injury or inflammation. Inhibition of $hPAR_1$ had no effect on platelet responses to cathepsin G, indicating a specific activation of PAR_4.

Patients with Hermansky-Pudlak syndrome, an autosomal recessive disorder, lack platelet dense granules and have no ADP autocrine response. However, these patients show only a mild bleeding phenotype (331, 332). It was hypothesized that the defect of ADP autocrine response was compensated by signaling through PAR_4 because the activation of this receptor occurs well after ADP release in normal individuals (333). Therefore, the ADP-autocrine response does not seem to be necessary for platelet aggregation as long as PAR_4 is strongly activated. However, it was observed by another laboratory that PAR_4-induced, but not PAR_1-induced, aggregation was entirely ADP-dependent using a specific AP for PAR_4 (334). Moreover, the authors found that subthreshold concentrations of an AP-activating PAR_1 potentiated the effects of a PAR_4-AP to stimulate maximal aggregation. In addition, both prostacyclin (PGI_2) and S-nitroso-glutathione, an NO-releasing agent, reduced AP-stimulated aggregation and fibrinogen-receptor up-regulation.

$PAR_4^{-/-}$ mice had markedly increased bleeding times (40). In addition, the platelets of these mice failed to change shape, mobilize calcium, or aggregate in response to thrombin. Ma *et al.* (118) observed that a specific AP for PAR_4 could induce endostatin release in rat platelets. A selective PAR_4 antagonist prevented endostatin release. In human platelets, specific agonists for PAR_1 or PAR_4 stimulated thromboxane production (117). Thromboxane produced by the combined stimulation of PAR_1 and PAR_4 was additive, suggesting the presence of two pathways for thrombin-induced thromboxane production in platelets. Blocking PAR_1 function with a domain-specific antibody resulted in substantial inhibition of thrombin signaling. However, PAR_4 can mediate platelet activation in response to high concentrations of thrombin (128). $hPAR_4$ itself showed no pharmacological effect at either 1 nM or 30 nM thrombin. However, blocking of both $hPAR_1$ and $hPAR_4$ resulted in a profound inhibitory response even at high concentrations of the proteinase (128). This shows that $hPAR_4$ activation is not necessary for robust responses in platelets when $hPAR_1$ function is intact. Thus, inhibition of $hPAR_1$ alone is probably not sufficient when seeking new antithrombotic therapies; it might be necessary to block both receptors simultaneously (106, 128). $hPAR_4$ seems to play a role as a backup signaling device that might mediate thrombin signaling to distinct effectors or with different kinetics compared with PAR_1. It might also allow platelets to respond to proteinases other than thrombin. Moreover, both receptors might be able to directly interact with each other.

The fact that $mPAR_3$ alone does not result in thrombin signaling on mouse platelets showed that $mPAR_3$ functions as a cofactor that promotes cleavage and activation of PAR_4 at low concentrations of thrombin (39, 40). Interestingly, knocking out the *PAR*$_3$ or the *PAR*$_4$ gene leads to a similar degree of protection against thrombosis in mice (40, 325). In humans, both $hPAR_1$ and $hPAR_4$ may independently mediate thrombin signaling (20, 128, 177). Thus, the mouse system does not have a direct analog in the human platelet. However, $mPAR_3$ promoted cleavage and activation of $hPAR_4$ as effectively as $mPAR_4$. Thus, $hPAR_4$ can be "cofactored" by PAR_3 *in vitro*, but it remains unclear whether or not cofactors such as PAR_1 play a direct role in $hPAR_4$ activation *in vivo* (39).

This reservoir of multiple thrombin receptors including PAR_1, PAR_3, and PAR_4 may allow for a precise regulation of thrombin-induced inflammatory stimuli under different pathophysiological conditions and the fine-tuned induction of different signal transduction pathways.

In endothelial cells, thrombin induces a rapid but transient activation of endothelial cells by stimulating the secretion of PGI_2 or platelet-activating factor (PAF) and by inducing the production of cell adhesion molecules (P-selectin, E-selectin) (47, 69, 70). Thrombin and a nonselective AP also induce synthesis and release of cytokines from endothelial cells such as IL-1, IL-6, and IL-8 (71, 72). Moreover, they regulate the cytokine-independent expression of ICAM-1 and VCAM-1 on human endothelial cells and cause an increased adhesion of monocytes to endothelial cells. This effect can be diminished by blocking antibodies (anti-CD 18, anti-CD 49d) (73). In the past, the effects observed above had been attributed to the activation of PAR_1. However, a recent *in vivo* study has shown that thrombin-induced leukocyte rolling and adhesion to vascular walls is mediated via PAR_4 (74). Although thrombin can trigger the recruitment of leukocytes to sites of inflammation, the PAR_1 antagonist RWJ-56110 does not block this effect. In contrast, a PAR_4-AP is able to reproduce

the effects of thrombin on leukocyte rolling and adherence, indicating that this proinflammatory effect of thrombin is due to the activation of PAR_4 and not PAR_1 (74). Thus, PAR_4 appears to function in early events of inflammatory reaction, in terms of recruiting leukocytes to the site of injury.

In contrast to a potential participation of PAR_1 in stimulating the angiogenic process (335), PAR_4 may play an opposing role. Apart from its antiangiogenic role via the activation of platelets, PAR_4 can also affect the arterial system by stimulating vascular smooth muscle mitogenesis (220).

PAR_4, like PAR_1 and PAR_2, also appears to be linked to signal transduction processes that modulate airway smooth muscle tone, because a PAR_4-AP caused a rapid transient contractile response in a murine tracheal preparation. This contraction was followed by a transient relaxant phase. The underlying molecular mechanism is still unclear, but the relaxation appears to be dependent on PAR_4-stimulated and COX-2-generated PGE_2 production, which activates the E-type prostanoid 2 receptor (152, 153).

In conclusion, preliminary data strongly suggest an important pathophysiological role of PAR_4 in vascular homeostasis and platelet function. However, the role of PAR_3 as a signaling molecule and the precise function of PAR_4 during inflammation and immune response remain to be clarified.

B. Signaling by proteinases via PAR_3 and PAR_4

PAR_3 and PAR_4 are the most recent members of the proteinase-activated receptor family. They are activated by thrombin. Subsequent to the cloning of PAR_3 (18) and PAR_4 (19, 20), relatively few publications have appeared examining the involvement of these receptors in signaling cascades. One puzzle that is yet to be resolved, as already mentioned, is the inability of PAR_3 to signal in response to its tethered ligand-derived peptide or to thrombin (39, 40). Until this issue is resolved unequivocally, a meaningful discussion about PAR_3 signaling is not possible. In contrast, PAR_4 appears to be capable of activating both G_{12}/G_{13} and G_q pathways (201, 336, 337).

As mentioned above, PAR_4 appears to be involved in the same signaling cascades as PAR_1 in human platelets. In contrast, mouse platelets express PAR_3 and PAR_4, but only PAR_4 appears to serve as a real signaling receptor, and PAR_3 serves solely to facilitate cleavage of PAR_4 by thrombin (39).

Studying downstream signaling events in which PAR_1 and PAR_4 could be involved in VSMC, Bretschneider *et al.* (220) revealed that these receptors have distinct downstream signaling kinetics. Later, activation of MAPKs after stimulation of PAR_4 was demonstrated. In their recent work, Sabri *et al.* (338) investigated PAR_4-mediated signaling in cardiomyocytes derived from $PAR_1^{-/-}$ mice. Using AYPGKF-NH_2, a modified PAR_4 agonist with an increased binding potency to PAR_4, they were able to demonstrate p38 phosphorylation as well as slight activation of PLC and ERK1/2. Additionally, thrombin and PAR_4-AP, but not PAR_1-AP, were able to activate Src in these cells, clearly indicating that the action of thrombin on Src activation is mediated by PAR_4, and not by PAR_1 in these cells. Further studies implicated the involvement of Src and EGFR kinase activity in the PAR_4-dependent p38 signaling pathway (338).

Recently, the involvement of PAR_4 in factor Xa-mediated signaling has been demonstrated. Camerer *et al.* (25) revealed that expression of PAR_1, PAR_2, and PAR_4 in *Xenopus* oocytes confers calcium signaling in response to factor Xa. They further showed that PAR_4-AP (AYPGKF-NH_2) stimulated increased phosphoinositide hydrolysis in endothelial cells and that responses to AYPGKF-NH_2 are absent in $PAR_4^{-/-}$ endothelial cells (240). Accordingly, PAR_4-mediated phosphoinositide hydrolysis in response to factor Xa has been clearly demonstrated (25).

V. Conclusions

There is no doubt that PARs play an important regulatory role during inflammation and immune response. Recent findings consolidate the concept that serine proteinases act as autocrine, paracrine, or endocrine mediators that talk directly to cells (13–15, 30). Some of these proteinase-stimulated effects are mediated through activation of proteinase-activated receptors, resulting in signal transduction pathways that are involved in inflammation and immune response. In many cases, PARs appear to play a proinflammatory role due to activation of proinflammatory mediators and cytokines (28, 59, 67, 72, 77–90, 94, 99, 263, 272, 274, 282, 283, 295, 339–345). In other instances, a protective and antiinflammatory role of PARs has been observed (52, 292, 298, 312, 346–348). Various serine proteinases, which serve as PAR activators, are relevant mediators during inflammation (Table 2). However, the precise role of PARs and serine proteinases in different tissues, cell types, and states of inflammation remains to be determined.

Open questions include: 1) For the several proteinases that are released in various tissues from different cell types during inflammation, what is their mechanism of regulation and what is their functional relevance? 2) What is the significance of the existence of multiple receptor subtypes for one proteinase, and how might these common proteinase targets be differentially regulated? 3) Which immune cells express functional PARs in humans *in vivo*, and what is their biological relevance? 4) Which are the endogenous proteinases that activate PAR_2 in human inflammation *in vivo*? 5) Which factors influence the regulation of PARs during inflammation or host defense? 6) Which effects of PAR-activating proteinases are non-PAR-mediated effects? 7) Which are the cell-specific signaling pathways and molecular mechanisms (transcription factors) after selective PAR activation in different inflammatory states and tissues? 8) What role do PARs play in neuronal transmission in humans? 9) Can PAR agonists or antagonists be used as therapeutic agents during inflammation or immune response in human diseases?

Thus, an integrative understanding of a regulatory role of serine proteinases as extracellular degradative enzymes as well as hormone-like signaling messengers in part via PARs and their counterregulation by extracellular proteinase inhibitors and intracellular molecules should lead to effective therapeutic approaches for various inflammatory/immune diseases such as thrombosis, sepsis, bacterial infections, gin-

givitis, asthma, hyperreactivity reaction, lung fibrosis, renal inflammation, rheumatoid arthritis, colitis ulcerosa, Crohn's disease, pancreatitis, Alzheimer's disease, amyotrophic lateral sclerosis, HIV encephalitis, atopic dermatitis, contact dermatitis, rosacea, wound repair, and infertility, for example, as well as pathophysiological symptoms such as pain and pruritus, in the future.

Acknowledgments

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