Research Overview

Upregulation of Proteinase-Activated Receptors (PARs) and Cardiovascular Function

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ABSTRACT  Proteinase activated receptors (PARs) have attracted particular attention for their possible role in cardiovascular physiology and disease. The aim of this article is to review what is known up to now about the upregulation of PARs that are targets for thrombin (PARs 1, 3, and 4) and PAR2, which is not activated by thrombin. Particular emphasis will be given to what is the “state of the art” on the upregulation of these receptors within the cardiovascular system and their relationship to those phenomena that can be encompassed under the term “cardiovascular inflammation.” Drug Dev. Res. 60:20–23, 2003.

Key words: proteinases; upregulation; cardiovascular

THE THROMBIN RECEPTOR FAMILY: PAR₁, PAR₃, and PAR₄

PAR₁

Proteinase activated receptor 1 (PAR₁), the first member of the PAR family to be cloned (also known as thrombin receptor), has been shown to play an important role in the cardiovascular system. Here, we will to review what is known about its upregulation, with particular emphasis on the cardiovascular system. Overall from the data present in the literature, upregulation of PAR₁ expression in the cardiovascular system appears to be a phenomenon less important than upregulation of PAR₂. One of the first studies that has dealt with this specific feature was performed by Nystedt et al. [1996] using cultured human umbilical vein-derived endothelial cells. This group showed that stimulation of cultured endothelial cells with the cytokines, tumor necrosis factor α (TNFα), and interleukin-1β (IL-1β) or with bacterial lipopolysaccharide (LPS) leads to an elevated expression of PAR₂ in a concentration-dependent manner. Interestingly, the time course of receptor induction after cytokine stimulation in these experiments was similar to those published for the upregulation of the adhesion molecules, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1. After 20 hours of stimulation, PAR₂ mRNA and protein levels were increased 5- to 10-fold over basal values. In the continued presence of TNFα, PAR-2 mRNA expression was found to remain elevated for up to 4 days. In contrast, the thrombin receptor was not upregulated by any of these inflammatory mediators. After this pioneering study, other work has evaluated the possible upregulation of PAR₁ compared with PAR₂. In isolated human coronary artery rings (HCA), exposure to IL-1β (1 ng/mL, 12 hours) or TNFα (3 nmol/L, 12 hours) did not affect PAR₁ expression, but increased PAR₄ mRNA levels by approximately fourfold as determined by quantitative polymerase chain reaction analysis. This study was the first to demonstrate functional PAR₄ in...
human arteries in situ, suggesting that upregulation of PAR1 may play a role in cardiovascular inflammation [Hamilton et al., 2001].

To date, few studies have reported examples of an upregulation of PAR1, although a number of such reports have described a lack of PAR1 upregulation. Recently, Wang and et al. [2002] have demonstrated that PAR1 immunoreactivity and transcript are increased in vascular smooth muscle cells in irradiated rat intestine. Intestinal irradiation increased PAR1 mRNA twofold, and caused a dose-dependent, sustained deficiency of microvascular thrombomodulin. This reduction in thrombomodulin, like the increase in PAR1, was associated with the severity of radiation toxicity.

In the context of cardiovascular function, there is an interesting report on the role of the thrombin receptor family in brain ischemia [Striggow et al., 2001]. In the study by Striggow [2001], it has been shown that PAR1 is significantly expressed in the brain, in particular in hippocampus, cortex, and amygdala. The highest densities of PAR3 were observed in the hippocampus, cortex, amygdala, thalamus, hypothalamus, and striatum and, apart from the striatum, a similar localization was found for PAR4. Within the hippocampal formation, each PAR subtype was localized predominantly in the pyramidal cell layers. When hippocampal slice cultures were exposed to a severe experimental ischemia (oxygen–glucose deprivation), the expression of PAR1 and PAR3 was upregulated with PAR2). These authors performed an in vitro analysis of human PAR1 promoter function, showing that a positive regulatory element is present within −4.2 and −3.2 kb of the transcription start site. This element has been examined in transgenic mice containing either 4.1 or 2.9 kb of the 5′ flanking PAR1 promoter sequence driving a LacZ reporter gene. Only the 4.1-kb PAR1 transgene was expressed in vivo and only during embryonic development. Strikingly, expression of the transgene was observed only in developing arteries and not in veins. Further examination of this putative regulatory sequence identified a novel noncoding RNA (ncR-uPAR) upstream of the PAR1 gene at −3.4 kb. The ncR-uPAR was able to upregulate a PAR1-core promoter-driven luciferase activity and to upregulate mRNA expression in vitro in a Pol II-dependent manner. This noncoding RNA appears to act in trans, albeit locally at the adjacent PAR1 promoter. These data suggest that the thrombin receptor plays a more important role in arteries as opposed to veins, and also that this untranslated RNA may play a role in PAR1 gene expression during embryonic development.

**PAR2**

The first demonstration of an upregulation of PAR2 came from the work of Nystedt et al. [1996], who showed that PAR2 was strongly upregulated by TNFα, IL-1α, and bacterial lipopolysaccharide as already outlined above. After this early finding, it was demonstrated that PAR2 is upregulated in vivo in the arterial and venous tissue of endotoxemic rats [Cicala et al., 1999]. Indeed, it was shown in rats that the intravenous administration of SLIGKV (0.1, 0.3, and 1 mg/kg) causes dose-dependent hypotension, and that in animals treated with LPS (20 hours earlier), there is an increased sensitivity to SLIGKV in terms of the hypotensive response. In particular, SLIGKV-induced hypotension in endotoxemic rats is already maximal at a concentration (30 μg/kg) that has only a very small effect in control rats. In this study, it was also shown that PAR2 can be localized using immunohistochemistry in the endothelial and smooth muscle cells in the aorta and jugular vein in LPS-treated rats. In keeping with the immunohistochemical detection of increased amounts of receptor protein, increased levels of PAR2 mRNA were also found using reverse transcription-polymerase chain reaction analysis.

In relation to the data described above, it was recently shown recently that the phosphodiesterase inhibitor, IBMX, attenuates the upregulation of PAR2 in endotoxemic rat aorta [Kawabata et al., 2001]. As mentioned above, upregulation of PAR2 can also be observed in isolated HCA's [Hamilton et al., 2001]. In HCA rings, the selective PAR1-activating peptide, TFLLR (0.01–10 μmol/L), caused an endothelium-dependent relaxation of precontracted preparations, whereas little or no change in vascular tension was elicited by the selective PAR2 agonist, SLIGKV, up to 100 μmol/L. On the other hand, exposure of HCA's to the cytokines, IL-1α (1 ng/mL, 12 h) or TNFα (3 nmol/L, 12 h) increased PAR2 levels by approximately fivefold, as determined by quantitative polymerase chain reaction analysis. Although IL-1α treatment did not affect the relaxation response caused by the selective PAR1 agonist, TFLLR-amide, there was an appearance of a significant endothelium-dependent relaxation in response to the selective PAR2 agonist, SLIGKV. These studies were the first to demonstrate
an upregulation of functional PAR2 in human arteries in situ, and suggest that this receptor may play a role in those situations, such as atherosclerosis, where there is vascular injury or inflammation.

Similar results that further support the hypothesis that PAR2 can be “unmasked” by an inflammatory cardiovascular event were obtained by using an animal model of balloon vascular injury [Damiano et al., 1999]. PAR2 expression was evaluated in rat carotid arteries subjected to balloon-catheter injury followed by perfusion fixation 1, 3, 7, or 14 days after injury. PAR2 was visualized immunohistochemically using a polyclonal antibody raised against the N-terminal residues 37–53 of human PAR2. In normal vessels, PAR2 labeling was strongest in adventitial myofibroblasts labeled strongly for PAR2. At 7 and 14 days after injury, the media and neointima of injured vessels showed an increase in PAR2 labeling; this labeling was most intense at the luminal edge of the neointima. In the same study, by using double immunohistochemical labeling, it was shown that the greatest expression of PAR2 was in areas where there was an increased proliferation as determined by measuring density of proliferating cell nuclear antigen–positive cells. In addition, PAR2 mRNA localization using in situ hybridization paralleled PAR2 expression. These data have clearly shown that there is an upregulation of PAR2 in response to vascular injury. Receptor upregulation is associated with medial smooth muscle damage, proliferating adventitial myofibroblasts, and smooth muscle cells of the neointima, particularly those at the proliferating luminal edge of the neointima. The data clearly imply a role for PAR2 upregulation in the response to vascular injury. The hypothesis that the upregulation of PAR2 is a physiologic response to an injury has been evaluated in a rat experimental model of myocardial ischemia–reperfusion injury. With the use of a Langendorff perfused rat heart model, it has been shown that after SLIGRL-NH2 infusion, there is a significant recovery of myocardial function and decrease in oxidation upon reperfusion after a period of ischemia. Furthermore, the administration of the selective PAR2 agonist decreased both the ischemic risk zone and the release of creatine kinase. All of these protective responses are coupled to an increased expression of PAR2 and TNFα in both nuclear extracts and whole heart homogenates.

The data suggest that an upregulation of PAR2 may constitute an early protective mechanism [Napoli et al., 2000]. More recently, this “beneficial effect” has been also confirmed by Milia et al. [2002] using a murine model of hind-limb ischemia. The involvement of PAR2 was assessed by administering SLIGRL-NH2 (at 300 and 1.5 nmol intramuscular daily for 21 days) in mice subjected to unilateral limb ischemia. SLIGRL-NH2 was also found to increase capillarity in perfused adductor skeletal muscles of normal rats, whereas the reverse peptide (LRGILS-NH2) was inactive. Interestingly, SLIGRL-NH2 enhanced the reparative angiogenic response to limb ischemia, resulting in an accelerated hemodynamic recovery and enhanced limb rescue, whereas the reverse peptide was inactive.

**CONCLUSIONS**

At this moment in time, we believe that we are at or just a few meters ahead on the starting gate in terms of unraveling the pathophysiologic role that PARs may play in the cardiovascular system. However, both the thrombin family of proteinase receptors (PAR1, PAR3, and PAR4) as well as PAR2 are bound to become increasingly recognized as time goes by in terms of their impact on cardiovascular disease. When specific pharmacologic tools, such as selective inhibitors as well as high-potency agonists for these receptors become available, it will be possible to understand and dissect further their pathophysiologic role in the cardiovascular system. The idea we want to leave with the reader is that although the thrombin family (PARs 1, 3, and 4) is very important in the signaling cascade in both physiologic and pathologic cardiovascular situations (as discussed elsewhere), PAR2 may play a major role in the cardiovascular system not necessarily under normal physiologic conditions, but rather in those pathologic conditions of the cardiovascular system where endothelial damage has occurred. It is an open question as to whether the upregulation of PAR2 in cardiovascular diseases is a pathologic event to be corrected (antagonists), or is a “beneficial” event (associated with a pathologic status), which is intended to counterbalance a compromised endothelium. There is no doubt in our minds that PAR2 will be found to be of importance in the setting of endothelial damage, which is a key trigger of the many events that characterize “cardiovascular inflammation.”

**REFERENCES**


PARs AND UPREGULATION


