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Protease-activated receptor (PAR)-1 and PAR-2 protect rat astrocytes from apoptotic cell death via differentially regulating JNK isoform-specific release of the chemokine GRO/CINC-1

Abstract

Protease-activated receptors (PARs), a subfamily of G protein-coupled receptors, are abundantly expressed in rat astrocytes. PARs are activated by certain proteases, like thrombin, trypsin and tryptase, through a unique mechanism that involves irreversible cleavage of the receptor and exposure of a new N-terminal domain acting as a tethered ligand. PAR-1, PAR-3 and PAR-4 are thrombin receptors, while PAR-2 is activated by serine proteases trypsin and mast cell tryptase, etc. In addition to the mitogenic role of PAR-1 on astrocytes established by our group previously, here we investigate whether different PARs, especially PAR-1 and PAR-2, play a role in regulating chemokine or cytokine release and whether they can exert a protective role under the ceramide-mimiced cell pathological condition.

In the present study, we report that thrombin, thrombin receptor agonist peptide (TRag) and PAR-2 activating peptide (PAR-2AP, SLIGRL) concentration-dependently upregulated the secretion of the chemokine growth-regulated oncogene/cytokine-induced neutrophil chemoattractant-1 (GRO/CINC-1), a functional counterpart of human interleukin-8, from primary rat astrocytes. However, treatment with either PAR-3AP (TFRGAP) or PAR-4AP (GYPGKF) failed to increase GRO/CINC-1 mRNA level.

Because activation of PAR-1 and PAR-2 both resulted in the release of the chemokine GRO/CINC-1 from rat astrocytes, we investigate whether the two PAR receptor subtypes can signal separately. By both ELISA and immunoblotting detection, it was found that PAR-1-induced GRO/CINC-1 release was mediated by protein kinase C (PKC), phosphatidylinositol 3 kinase (PI3K) and mitogen-activated protein kinase kinase 1/2 activation, whereas these three kinases were not involved in PAR-2-induced GRO/CINC-1 release. Extracellular signal-regulated kinase 1/2 seemed to be only partially involved in the GRO/CINC-1 secretion induced by PAR-1, but not by PAR-2. Despite such clear differences between PAR-1 and PAR-2 signaling pathways, c-Jun N-terminal kinase (JNK) was identified in both signaling pathways to play a pivotal role. In contrast, p38 mitogen-activated protein kinase was not involved in either PAR-1 or PAR-2 signaling pathway.

Although JNK played a pivotal role in both PAR-1 and PAR-2 signaling pathways, PAR-1 and PAR-2 activated different JNK isoforms. PAR-1 induced the phosphorylation of both 46 kDa and 43 kDa JNK isoforms, whereas PAR-2 caused only 43 kDa JNK phosphorylation. Unlike PAR-1-induced JNK activation, which was mediated by PKC and

PI3K, PAR-2-induced JNK phosphorylation was apparently independent of PKC. Moreover, only activation of PAR-1, but not PAR-2, led to c-Jun phosphorylation via JNK. All differences between the isoforms of JNK phosphorylation, JNK upstream activators and JNK effects on its downstream transcription factor c-Jun clearly indicate that different JNK isoforms with distinct properties might be involved in the PAR-1 and PAR-2 signaling pathways.

By JNK isoform-specific loss-of-function studies using small interfering RNA, we demonstrate that PKC-mediated JNK2 activation and PI3K-mediated JNK3 activation were essential for PAR-1-induced the chemokine GRO/CINC-1 secretion, whereas PAR-1-mediated JNK1 activation was mainly responsible for c-Jun phosphorylation, which was not involved in GRO/CINC-1 release. In contrast, PAR-2-induced JNK1 activation, which failed to phosphorylate c-Jun, uniquely contributed to GRO/CINC-1 release. Therefore, our results for the first time show that JNK-mediated chemokine GRO/CINC-1 release occurs in a JNK isoform-dependent fashion and invokes PAR subtype-specific mechanisms.

Further studies demonstrate that activation of PAR-1 and PAR-2 as well as application of the recombinant GRO/CINC-1 protected astrocytes from C_2-ceramide-induced cell death. Protection occurred with suppression of cytochrome c release from mitochondria. The inhibition of cytochrome c release was largely reduced by the antagonist of chemokine receptor CXCR2, SB-332235. Importantly, the specific JNK inhibitor SP600125 significantly abolished the protective action of PAR-1. These results for the first time demonstrate that PAR-1 plays an important anti-apoptotic role in brain by regulating the release of the chemokine GRO/CINC-1, which gives a feedback through its receptor CXCR2 to preserve astrocytes from toxic insults. This novel mechanism is likely to explain the protective action of thrombin at low concentrations after brain injury. So far, the protective effect of PAR-2 in brain has not received great attention. Our results here in this context are of high significance showing that PAR-2 signaling pathway could be another additional protective pathway in brain via regulating the release of the chemokine GRO/CINC-1.

Our results suggest that PAR-1 and PAR-2 have overlapping functions, but can activate separate pathways under certain pathological conditions, to rescue neural cells from cell death. Different JNK isoforms play pivotal roles in the PAR-induced cell protection via regulating the chemokine GRO/CINC-1 secretion. This provides new functional insights into PAR-JNK signaling and the protective actions of PARs in brain.