Lipoxygenases (LOXs) are nonheme iron-containing enzymes that catalyze dioxygenation of free or esterified polyenoic fatty acids to the corresponding hydroperoxides. Although LOXs in plants have been known for almost a century, the first mammalian LOXs were not discovered until the mid 1970s. In 1984 a 15-LOX activity was described in human atherosclerotic lesions, and since then the number of reports connecting LOXs to the cardiovascular system has steadily increased. Cardiovascular research requires suitable animal models, with rats, mice, and rabbits as the preferred species. Unfortunately, concerning LOXs, there are profound differences between these animal species and humans. In humans there are 6 functional LOX genes that encode for separate LOX isoforms, but only 3 of them (5-LOX, platelet-type 12-LOX, reticulocyte-type 15-LOX) appear to be important for vascular disorders. Except for the 5-LOX gene (mapped to chromosome 10), all human LOX genes are located on the short arm of chromosome 17. In mice, however, there are 7 functional LOX genes, and they were mapped to synthetic regions of the murine genome. In contrast to humans, there is no reticulocyte-type 15-LOX in mice, but the leukocyte-type 12-LOX appears to be its functional equivalent. This difference needs to be considered if the enzymes exhibit their biological activities via the formation of bioactive arachidonic acid oxygenation products (12S-HETE in mice versus 15S-HETE in humans). In rabbits, the situation is even more complex. Here, 2 separate genes have been discovered encoding for a reticulocyte-type 15-LOX and a leukocyte-type 12-LOX. Expression of the 2 genes is under the control of distinct regulatory sequences, and their tissue-specific expression patterns are also quite different. Similar species-specific differences between orthologous LOX isoforms have also been described for the human 15-LOX2, which is an arachidonate 8-LOX in mice, and for the murine epidermis-type 12S-LOX (no functional human gene). These differences indicate that care should be taken if experimental data on LOX metabolism in the cardiovascular system are being transferred from one species to another.

LOX activities in a cellular system are tightly controlled on transcriptional, translational, and posttranslational levels. In human monocytes/macrophages, expression of the reticulocyte-type 15-LOX is induced by Th2-cytokines, and the intracellular signal transduction cascade involves functional cell surface receptors, various types of protein kinases, and transcription factors of the STAT family, as well as activation of cellular acetyl transferases modifying the histone structure. Granulocyte-macrophage colony-stimulating factor (GM-CSF) increases production of the 5-LOX product leukotriene B4 in human neutrophils. Nuclear transcription assays indicated that the rate of 5-LOX gene transcription was augmented several times, whereas the half-life of the corresponding mRNA was not altered. These data suggest transcriptional mechanisms in the regulation of 5-LOX gene expression, but the mechanistic details of this effect are not completely understood. In rabbit reticulocytes, translation of the 15-LOX mRNA is suppressed by regulatory proteins (hnRNPs K and E1), and this translational silencing appears to be important for cell maturation. Expression of 5-LOX is also regulated on the mRNA level. In Mono Mac 6 cells, transforming growth factor and vitamin D3 modify maturation of 5-LOX transcripts, which leads to augmented expression of the functional enzyme. Posttranslational mechanisms for the expression of various LOX isoforms have also been investigated. Treatment of human neutrophils with the calcium ionophore A23187 induces activation of the 5-LOX pathway and translocation of the cytosolic enzyme into the nucleus. Moreover, the 5-LOX protein can be phosphorylated by MAPK-activated protein kinase-2, and this phosphorylation appears to correlate with an increased enzymatic activity. Another element of posttranslational regulation of the 5-LOX pathway is the 5-LOX activating protein (FLAP). Although the detailed mechanism of FLAP-mediated 5-LOX activation remains unclear, it may be related to augment substrate availability.

In this issue of Circulation Research, Coffey et al. used human platelets as a model system to define acute agonist activation pathways for the platelet-type 12-LOX. This LOX isoform was the first mammalian LOX discovered in 1974, but its biological relevance is still unclear. Collagen and collagen-related protein (CRP) acutely induce 12-H(p)ETE synthesis via activation of glycoprotein VI-dependent (GPVI) signaling cascade. Using specific inhibitors of intracellular signaling, the authors investigated the mechanistic basis for this stimulatory effect and found that upregulation of 12-H(p)ETE biosynthesis involves activation of src-tyrosine kinases, of PI3-kinase, and an increase in the intracellular calcium concentration. The GPVI signaling cascade has previously been characterized in platelets, but it has never been linked to activation of any LOX isoform. Hence, this study may represent a “template” for acute activation of this...
Collagen-induced signaling events leading to the acute activation of platelet 12-LOX. Collagen or collagen-related peptide (CRP) binds to clusters of glycoprotein VI (GPVI) receptors on the platelet surface. This binding induces rapid association and phosphorylation of the GPVI-associated Fcrγ-chain by src-tyrosine kinases. Subsequent activation of PI3-kinase and stimulation of intracellular Ca2+ pools precedes activation of p12-LOX. The negative regulatory roles of platelet endothelial cell adhesion molecule 1 (PECAM-1) and protein kinase C (PKC) are highlighted.

and/or other LOX isoforms in different cellular systems. Another interesting finding is the sensitivity of the 12-LOX pathway toward activation of platelet endothelial cells adhesion molecule (PECAM-1). Although PECAM-1 has already been implicated in platelet function, its biological importance is far from clear. Coffey et al indicated for the first time that PECAM-1 might allow for attenuation of GPVI-mediated 12-LOX activity. This regulatory effect may impact platelet physiology and alter aggregation behavior of the cells. The authors also report that specific inhibitors of protein kinase C (PKC) strongly activate CRP-induced 12-H(p)ETE formation. From the physiological point of view, it may confer an evolutionary advantage to the cells if an acute activation cascade has a number of "braking mechanisms" that temper the regulatory extent and prevent run-away activity. For the 12-LOX pathway of human platelets, PECAM-1 and PKC may represent such inverse regulatory mechanisms, which are aimed at achieving a steady state of 12-H(p)ETE formation under the conditions of cell activation.

In the Figure, the CRP-induced signal transduction cascade leading to upregulation of 12-H(p)ETE formation is summarized. Binding of collagen or CRP to GPVI activates src-tyrosine kinases, which may be counteracted by PECAM-1. Furthermore, the authors implicated PI3-kinases and increased cytosolic calcium levels in the signal transduction cascade, but the precise order of the activation events downstream of the src-tyrosine kinases has not been clarified. A major advantage of the model system used in the Coffey et al study is that it does not require exogenous arachidonic acid to elicit LOX activity. Thus, the system includes the rate-limiting step of the arachidonic acid cascade, the liberation of fatty acid substrates from internal lipid stores. In eicosanoid research, this reaction step is frequently shunted by applying exogenous arachidonic acid. In the study by Coffey et al, endogenous substrates are used. Thus, the system also allows investigations on fatty acid liberating enzymes. Unfortunately, the authors did not attempt to identify the ester lipid hydrolyzing activities involved in CRP-dependent upregulation of 12-H(p)ETE formation. The observation that thrombin, which upregulates the activity of certain phospholipase A2 (PLA2) isoforms, does not activate the 12-LOX pathway suggests either that thrombin activates a PLA2-isoform that does not route arachidonic acid to platelet 12-LOX or that additional regulatory elements may be required, which thrombin simply does not trigger. Another point of criticism is that the mechanistic conclusions drawn in this article are exclusively based on pharmacological intervention studies. The use of genetically modified animals and/or other alternative strategies, which lead to impaired expression of certain constituents of the CRP-induced signaling cascade, would have considerably strengthened the mechanistic conclusions. Nevertheless, this study represents a detailed analysis of acute posttranslational activation of a mammalian lipoxygenase and poses intriguing questions as to the nature of platelet-type 12-LOX activation within the context of collagen exposed from the subendothelial matrix during vascular insult.

References


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