PERSPECTIVE

The Yin and the Yang of 5-Lipoxygenase Pathway Activation

TIMOTHY D. BIGBY

Department of Medicine, VA San Diego Healthcare System and the University of California, San Diego

This article is available online at http://molpharm.aspetjournals.org

The 5-lipoxygenase pathway is the source of potent pro-inflammatory mediators. More than 20 years ago, the details of the 5-lipoxygenase pathway began to emerge from seminal work performed by Borgeat and Murphy while they were working in the laboratory of Bengt Samuelsson (Borgeat and Samuelsson, 1979; Murphy et al., 1979). A vast body of evidence has subsequently accrued regarding the cells of origin of these metabolites and their biological importance. The 5-lipoxygenase pathway is one of at least four lipoxygenase pathways of arachidonic acid metabolism (Brash, 1999). The 5-lipoxygenase acts preferentially upon unesterified arachidonic acid, inserting molecular oxygen at the fifth carbon and forming the hydroperoxyl intermediate, 5-hydroperoxyeicosatetraenoic acid (Bigby et al., 1998; Funk, 2001). This same enzyme then catabolizes a dehydration reaction, forming the unstable epoxide intermediate, leukotriene (LT) A_4. In intact inflammatory cells, the presence of the 5-lipoxygenase activating protein is required to make this enzyme active (Dixon et al., 1990). LTA_4 can then be further metabolized to LTB_4 by LTA_4 hydrolase or to LTC_4 by conjugation of glutathione at the sixth carbon by the action of LTC_4 synthase. Additional studies established that LTC_4 and its extracellular metabolites LTD_4 and LTE_4 are the constituents of slow-reacting substance of anaphylaxis, but they are now more properly termed cysteinyl leukotrienes. The cysteinyl leukotrienes have been recognized to mimic many of the clinical manifestations of asthma, including sustained bronchoconstriction, hypersecretion of mucus, and airway edema. LTE_4 and its metabolites can induce eosinophil chemotaxis. By contrast, LTB_4 induces chemotaxis, inflammatory cell stimulation, and a complex role in host defense, suggesting that it is a key component of the innate immune response (Bailie et al., 1996).

The term leukotriene was coined by Samuelsson and his colleagues to reflect their cells of origin, inflammatory cells, and the recognition that these compounds have a conjugated triene structure (Samuelsson, 1983). In fact, this pathway is largely restricted to cells of myeloid origin, although the components of the pathway expressed by a specific inflammatory cell type varies. For example, stimulated neutrophils synthesize LTB_4 but do not seem to have the capacity to synthesize LTC_4. By contrast, eosinophils synthesize LTC_4 almost exclusively (Bigby et al., 1998).

The regulation of this cell-specific expression and how it might be modulated in disease or used to therapeutic advantage have been the focus of significant investigation. 5-Lipoxygenase activity and product generation at sites of inflammation might be modulated in multiple ways. The most obvious would be the recruitment and stimulation of additional inflammatory cells. Another possibility is modulating the expression of the enzymatic components through transcriptional or post-transcriptional mechanisms. There is evidence that this occurs and this may play a role in chronic disease (Ring et al., 1996; Coffey et al., 1998). Modulation of signaling pathways leading to the activation of the pathway within the inflammatory cell, however, might provide more immediate and local control of product generation at inflammatory sites.

The earliest investigations identified that 5-lipoxygenase was activated by calcium, required ATP, and required phospholipid for optimum activity (Samuelsson, 1983). Subsequent studies demonstrated that 5-lipoxygenase is a cytosolic enzyme that translocates to the nuclear envelope upon activation (Woods et al., 1993). Moreover, we now recognize that 5-lipoxygenase may reside in the nucleus and can bind to chromatin. However, the function of this enzyme in the nucleus remains relatively obscure. The N-terminal β barrel of 5-lipoxygenase plays a key role in the translocation of this enzyme to the nuclear envelope (Chen and Funk, 2001). Thus, much of the work on the 5-lipoxygenase pathway has focused on structure-function relationships and anatomic location of the component enzymes with the cell and within the organism. Many studies of the 5-lipoxygenase pathway have been conducted using nonbiological stimuli of this pathway, primarily calcium ionophores, because of the ease of stimu-

ABBREVIATIONS: LT, leukotriene; PKA, protein kinase A; MAP, mitogen-activated protein.

lating the cells and the robust product generation that simplified the problem of measuring these products by complex biochemical techniques.

Naturally occurring, proinflammatory stimuli, such as platelet activating factor and formyl peptides, have been shown to stimulate inflammatory cells to synthesize and release small amounts of leukotrienes. Priming cells with cytokines or lipopolysaccharide can enhance this stimulated synthesis and release. Investigations examining the intricacies of these receptor-ligand interactions and the signaling pathways involved in activation of this 5-lipoxygenase product generation in inflammatory cells have been performed, although this work has lagged behind other areas of investigation in the field. Ligands initiate activation of tyrosine kinase activity probably via calcium release; that, in turn, activates p38 kinase, although the intervening steps are not elucidated (Figure 1). Work by Werz et al. (2000) at the Karolinska Institute demonstrates that mitogen activated protein (MAP) kinase-activated protein kinases 2 and 3 phosphorylate 5-lipoxygenase and that this phosphorylation is initiated directly by an upstream p38 MAP kinase (Werz et al., 2000). These authors also demonstrate that calcium is necessary but insufficient to activate 5-lipoxygenase and that direct phosphorylation is necessary for translocation of 5-lipoxygenase to the nuclear envelope. Moreover, phosphorylation of Ser271 in 5-lipoxygenase is enhanced by arachidonic acid and this phosphorylation is associated with translocation to the nucleus (Werz et al., 2002). Additional data implicate the ERK-MEK signaling pathway in translocation of 5-lipoxygenase (Boden et al., 2000). Cross talk between these pathways for 5-lipoxygenase activation has not been sufficiently considered or explored.

Agents that raise intracellular cAMP can down-regulate 5-lipoxygenase activity. Ham et al. (1983) were the first to demonstrate that PGE_2 inhibited leukotriene synthesis. Additional studies demonstrated that adenosine down-regulates stimulated 5-lipoxygenase activity in inflammatory cells via the A_{2a} receptor, presumably. In this issue of *Molecular Pharmacology*, Flamand et al. (2002) have made a significant contribution to their own prior work in this area and to our understanding of regulatory steps limiting 5-lipoxygenase activity. The authors have demonstrated that agents that increase cAMP in neutrophils inhibit translocation of 5-lipoxygenase when these cells are stimulated. The authors have induced increases in cAMP by a variety of pharmacological means. They have primed neutrophils with tumor necrosis factor-α/granulocyte macrophage-colony stimulating factor and have stimulated the cells with PAF or thapsigargin. In the presence of elevated cAMP, 5-lipoxygenase does not significantly translocate to a nuclear membrane fraction and this is correlated with a decrease in product formation as detected by HPLC. An inhibitor of phosphodiesterase IV, RO 20-1724 also inhibits translocation. The PKA inhibitors H89 and KT-5720 block the effects of agents that elevate cAMP. A p38 MAP kinase inhibitor, SB 203,580 inhibits 5-lipoxygenase activity in these cells. An adenosine receptor agonist inhibited p38 phosphorylation and 5-lipoxygenase translocation and a PKA inhibitor allows p38 phosphorylation and 5-lipoxygenase translocation in thapsigargin-stimulated neutrophils. These data provide convincing evidence that elevation of intracellular cAMP inhibits stimulated 5-lipoxygenase activity in neutrophils by inhibiting 5-lipoxygenase translocation. They also present a convincing case that this is mediated by direct effects of PKA on p38 MAP kinase in the signaling pathway.

Much more work remains to be done on signaling pathways for the activation of 5-lipoxygenase. Some key remaining questions are: 1) What are the signaling elements between the receptor and tyrosine kinase? 2) Which tyrosine kinase is involved? 3) What are the intervening steps between tyrosine kinase and p38 MAP kinase? 4) Is the ERK-MEK pathway a parallel pathway for activation and is there cross-talk between these two? With respect to the report by Flamand et al. (2002), additional work will help to confirm and solidify their findings. Although most data suggest the contrary, additional work should be done with more molecular tools to exclude 5-lipoxygenase as a direct substrate of PKA and exclude direct PKA inhibition of the enzyme. Additional site-
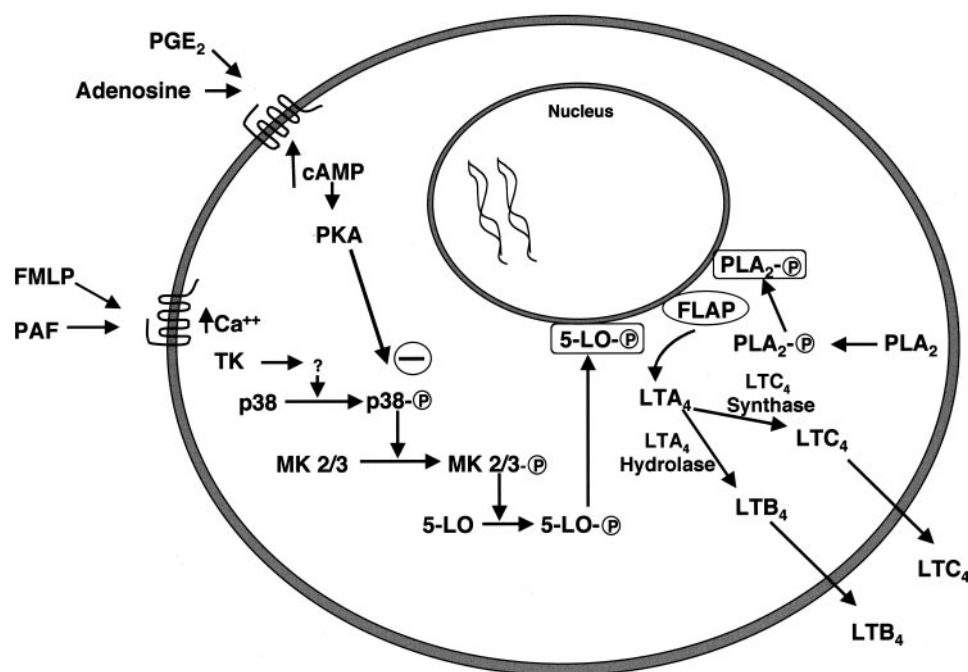

Fig. 1. Activation of 5-lipoxygenase in neutrophils. Receptor-mediated agonists stimulate a calcium flux and a tyrosine kinase (TK) is activated. TK, probably via intervening steps, phosphorylates p38 MAP kinase. p38 MAP kinase then phosphorylates MAP kinase-activated protein kinase (MK) 2 and 3, which then directly phosphorylate 5-lipoxygenase (5-LO), which in turn translocates to the nuclear envelope. Cytosolic phospholipase A_2 (PLA_2) is also phosphorylated and translocates to the nucleus. Translocated PLA_2 releases arachidonic acid that is then presented to 5-lipoxygenase by the 5-lipoxygenase activating protein (FLAP), resulting in the synthesis of leukotriene A_4 by two sequential enzymatic steps. Agonists that increase intracellular cAMP activate protein kinase A (PKA), which inhibits p38 MAP kinase activation, thus inhibiting 5-lipoxygenase translocation and activation. PKA may also phosphorylate 5-lipoxygenase directly; so far, the data do not support this.

directed mutagenesis of 5-lipoxygenase at putative PKA consensus sites might be helpful. Dominant negative strategies to inhibit PKA activation within the cell or other molecular targets would help elucidate not only the signaling pathway, but also the down-regulatory elements.

Why should we care about these studies? The lipoxygenase pathway generates products that are important in asthma, allergy, and inflammation. Modulation of this pathway by pharmacological agents that up-regulate cAMP may, in fact, contribute to the efficacy of existing drugs, such as methylxanthines or β_2-agonists, that are currently used to treat such disorders as asthma. However, such drugs suffer from a lack of specificity. By contrast, phosphodiesterase IV inhibitors, already in development, may offer the opportunity to inhibit inflammatory mediator pathways, such as the 5-lipoxygenase pathway, in a cell-specific fashion. Further elucidation of the signaling pathways for 5-lipoxygenase activity and its modulators may also help identify additional molecular targets for drug development.

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Address correspondence to: Dr. Timothy D. Bigby, Mail Code 111-J, VA San Diego Healthcare System, 3350 La Jolla Village Drive, San Diego, CA 92161. E-mail: tbigby@ucsd.edu