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Review

Transcellular biosynthesis of eicosanoid lipid mediators ☆

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ABSTRACT

The synthesis of oxygenated eicosanoids is the result of the coordinated action of several enzymatic activities, from phospholipase A_2 that releases the polyunsaturated fatty acids from membrane phospholipids, to primary oxidative enzymes, such as cyclooxygenases and lipoxygenases, to isomerases, synthases and hydrolases that carry out the final synthesis of the biologically active metabolites. Cells possessing the entire enzymatic machinery have been studied as sources of bioactive eicosanoids, but early on evidence proved that biosynthetic intermediates, albeit unstable, could move from one cell type to another. The biosynthesis of bioactive compounds could therefore be the result of a coordinated effort by multiple cell types that has been named transcellular biosynthesis of the eicosanoids. In several cases cells not capable of carrying out the complete biosynthetic process, due to the lack of key enzymes, have been shown to efficiently contribute to the final production of prostaglandins, leukotrienes and lipoxins. We will review in vitro studies, complex functional models, and in vivo evidences of the transcellular biosynthesis of eicosanoids and the biological relevance of the metabolites resulting from this unique biosynthetic pathway. This article is part of a Special Issue entitled "Oxygenated metabolism of PUFA: analysis and biological relevance".

1. Introduction

Arachidonic acid (AA) is an abundant polyunsaturated fatty acid of the membrane phospholipids, where it is stored in the sn-2 position of phosphatidylinositol and/or phosphatidylcholine. AA regulates the physical properties of the membranes but when released as free acid by phospholipases (PLs) it has an essential role as a precursor for enzymatic and non-enzymatic biosynthetic pathways leading to the production of eicosanoids, a large family of potent local hormones acting at nanomolar concentration in autocrine/paracrine way [1]. PLs are sensitive to the increase in intracellular free calcium concentration as a result of physical stimuli and can be activated by growth factors, hormones, cytokines and eicosanoids acting through specific receptors [2].

Several families of eicosanoids are produced from AA, but the main biologically relevant are: prostanoids (prostaglandins, PGs, prostacyclin, PGI_2 and thromboxane A_2, TxA_2), synthesized by the cyclooxygenase (COX) pathway (Fig. 1); leukotrienes (LTs, i.e. LTB_4 and cysteinyl-LTs), derived from 5-lipoxygenase (5-LO) pathway (Fig. 1); and lipoxins (LXs), originating from the interaction of 5-LO, 12-LO and 15-LO (Fig. 2).

The COX pathway was the first AA metabolic pathway elucidated. Two isoforms of COX have been identified: COX-1 is constitutively expressed in most cells and tissues, and is involved in vascular homeostasis, protection of the gastric mucosa and regulation of renal function; and the inducible isoform COX-2, which is expressed in response to inflammatory stimuli, even though it is constitutively present in some types of endothelial cells, brain and kidney [3]. The double catalytic activity of COX leads to the production of a highly unstable endoperoxide intermediate PGH_2, which is further metabolized by specific synthase enzymes to produce PGs (PGE_2, PGI_2, $PGF_{2\alpha}$, PGD_2) and TxA_2 (Fig. 1).

Prostanoids exert various biological functions in several cells and tissues by interacting with specific G-protein-coupled receptors (GPCRs), integral membrane proteins that transmit signals inside the cells (for a comprehensive review, see [4]). Prostanoid roles in physiology and pathophysiology were initially derived from the observation of the effect of aspirin [5,6] or by exogenous addition of each prostanoid, and lately by the characterization of knock-out mice phenotypes [7]. They are mainly involved in inflammation, pain, fever, platelet and vascular homeostasis, immunity, and reproduction [8].

The 5-LO pathway leads to the production of another large family of potent lipid mediators called LTs (Fig. 1), first discovered by Samuelsson et al. in rabbit polymorphonuclear leukocytes (PMN) [9,10]. Furthermore,

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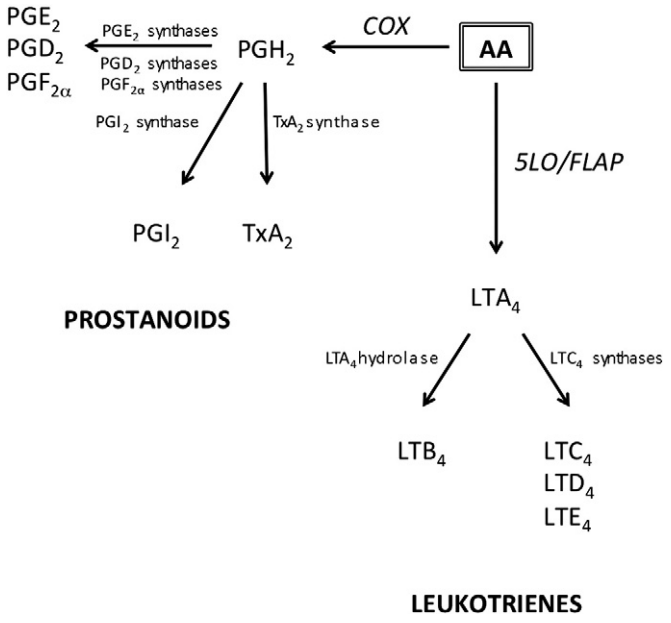

Fig. 1. Prostanoid and leukotriene biosynthetic pathways. Several proteins have been identified as possessing the ability to convert PGH_2 into PGE_2, PGD_2 or $PGF_{2\alpha}$ as well as to convert LTA_4 into LTC_4. FLAP (five-lipoxygenase activating protein) binds 5-LO on the nuclear membrane and contributes to handle AA to the 5-LO.

5-LO participates, in coordination with 12/15-LO, to the formation of LXs [11] (see Fig. 2 and the specific section below). 5-LO is expressed by cells of myeloid origin, particularly neutrophils, eosinophils, monocytes/macrophages, and mast cells [12–15], as well as in B lymphocytes [16]. To synthesize the highly unstable intermediate LTA_4, 5-LO requires the intervention of 5-lipoxygenase activating protein (FLAP) that, in intact cells, associates to the membrane and presents AA to 5-LO [17–19]. LTA_4 is subsequently metabolized by LTA_4 hydrolase into LTB_4 or conjugated with glutathione by LTC_4 synthase to yield the cysteinyl-LTs, i.e. LTC_4, LTD_4 and LTE_4 (Fig. 1). LTs interact with specific GPCRs ([20]) to display proinflammatory activities both in health and disease. LTB_4 is a potent chemotactic for neutrophils and eosinophils and plays a number of roles in inflammation and immune regulation [21]. Cysteinyl-LTs, which are known to increase vascular permeability and regulate smooth muscle tone, have a clear role in asthma and allergic rhinitis and have been implicated in other inflammatory conditions including cardiovascular, gastrointestinal, immune, and neurodegenerative diseases [22,23].

2. Initial evidence for transcellular metabolism: communication among platelets and ECs to produce PGI_2

Since the discovery of prostanoids, lipoxins and leukotrienes as AA derivatives, investigations focused on the identification of cells possessing all the enzymes involved in the individual steps of their biosynthesis. However, these investigations were soon paralleled by the observation that many cells were found to contain only the enzymes responsible for the biochemical conversion of unstable intermediates (such as LTA4 or PGH2) into the final bioactive compounds. For example, 5-LO lacks in platelets, endothelial cells (ECs) and vascular smooth muscle cells (VSMCs), but all these cells seemed to express secondary enzymes such as LTC_4-synthases capable to convert LTA4 into cysteinyl-LTs (Fig. 2).

Bunting et al. [24] were the first to postulate that an anti-aggregating compound (later named prostacyclin, PGI_2) was produced by the arterial wall with COX pharmacologically inactivated with indomethacin following incubation with activated platelet-rich plasma, suggesting that the unstable intermediate PGH_2 from platelets was converted by the arterial rings into the biologically active, anti-aggregating compound. This observation introduced the hypothesis that eicosanoid production could result from the cooperation of cells, one of which is activated to biosynthesize through a primary oxidative enzyme (COX or LO) an intermediate that is transferred to a neighboring cell to carry out the final synthesis of lipid mediators. The latter has no need of being activated and is impeded or lacks the ability to produce the intermediate but is able of converting it into biologically active metabolite(s) by the presence of secondary enzymes, such as prostaglandin isomerases, LTA_4 hydrolase or LTC_4 synthases (Table 1). We will see below that also the free AA could serve as a biosynthetic intermediate in this process (see Section 3). This mechanism of cell–cell cooperation is termed transcellular metabolism or transcellular biosynthesis and the seminal work by Bunting et al. indicates that this is a suitable event under normal conditions, where accumulation of platelets on the vessel should be

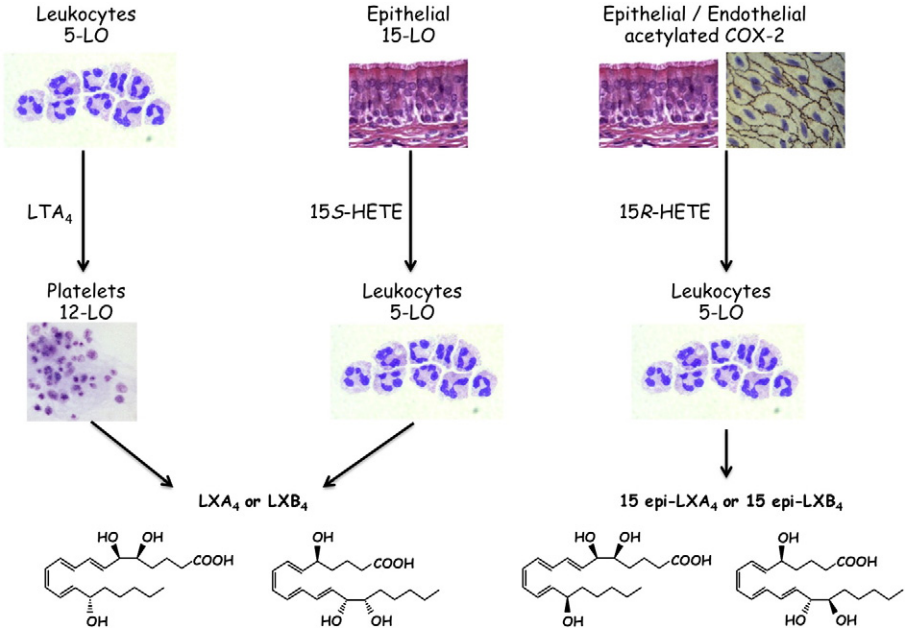

Fig. 2. Simplified schematic overview of the lipoxin biosynthetic pathways.