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COMMENTARY

INTERACTIONS BETWEEN PROSTAGLANDINS AND LEUKOTRIENES

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Studies on the metabolic conversion of arachidonic acid (AA) to prostaglandins (PGs) have occupied a prominent position in the biochemical literature over the last two decades. The bewildering array of biological activities attributed to these substances did not foster an easy understanding of their significance in cellular function. Reports showing PGs to be causal factors in inflammation [1, 2] led to the belief that their actions were pathological in nature. However, biochemical studies relating PG action to specific receptors linked to adenylate cyclase indicated that PGs may also play a role in regulating cellular activity via second messenger cyclic AMP [3]. Now it is well established that prostaglandins play important regulatory roles in kidney, stomach and circulatory processes, although our understanding of the full scope of their activities is by no means fully developed.

The recent finding that slow-reacting substance of anaphylaxis (SRS-A), a purported causal factor in asthma, is representative of a new class of AA oxygenation products, called the leukotrienes (LTs) [4], adds further to the scope of activities of metabolites of arachidonic acid (Fig. 1). SRS, which is a mixture of LTC_4 and LTD_4, representative of the peptidoleukotrienes (S-LTs), in addition to involvement in asthma appears to relate to other allergic responses [5]. There is also evidence of a role of LTD_4 in circulatory functions [6]. LTB_4 is another leukotriene (Fig. 1), all of which derive from the initial enzyme, 5-lipoxygenase. LTB_4 is a potent chemoattractant for neutrophils and a complete secretogue at higher levels [7]. Since the polymorphonuclear leukocyte (PMN) is essential for inflammatory processes, involvement of LTB_4 in this disease process is indicated [7, 8]. Thus, like the prostaglandins, the leukotrienes represent a group of potent AA oxygenation products whose actions appear to be largely pathogenic in nature. However, in view of the requirement for recruitment of phagocytic cells to areas of infection and the possible involvement of LTB_4 in these processes, it seems likely that, as with the prostaglandins, the leukotrienes may also have important regulatory roles in cell function.

Considering the many actions of the prostglandins and leukotrienes, some in mimicry and some in opposition to each other, it is not unreasonable to expect important interactions to occur between these two groups of compounds. This is more true in view of the fact that products of both pathways derive from the same precursor, arachidonic acid, and possibly even from the same substrate pool (Fig. 1). It is the purpose of this report to consider such interactions in detail.

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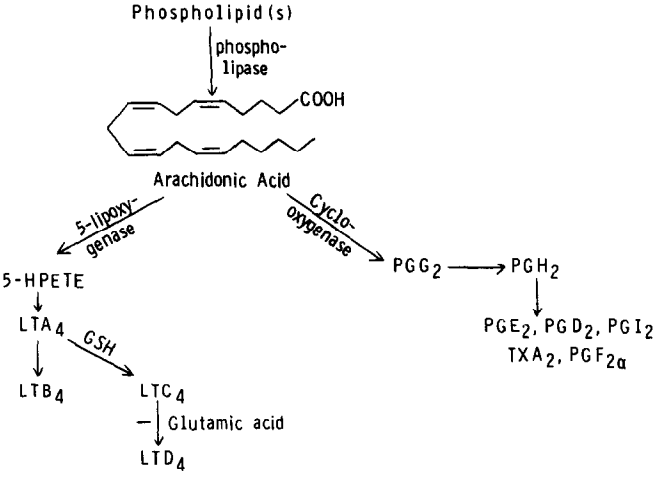

Fig. 1. Enzymatic conversion of arachidonic acid to products of the prostaglandin and leukotriene pathway.

Stimulatory effects of leukotrienes on prostaglandin synthesis

In 1969, Piper and Vane [9] reported that SRS-A released rabbit aorta contracting substance (RCS) from unsensitized guinea pig lungs. In light of the present knowledge that SRS-A is a mixture of LTC_4 and LTD_4 and that RCS is a mixture of PG endoperoxides and thromboxane A_2 [10, 11], this represents the first evidence of an interaction between leukotrienes and prostaglandins. However, an additional decade of research was required to establish this fact since the compositions of SRS-A and RCS were not known at that time. The actions of LTC_4 and LTD_4 (S-LTs) on the guinea pig lung are largely mediated by thromboxane A_2 (TXA_2) and thus are inhibitable by indomethacin [12]. Infusion of LTD_4 into the guinea pig confirmed the mediator role of TXA_2, but at higher levels of LTD_4 the contractile response was not totally inhibited by indomethacin [13]. When administered by insufflation, the action of LTD_4 was enhanced by indomethacin [13]. LTB_4 has also been shown to cause bronchoconstriction when administered either by intravenous or aerosol routes and appears to be totally dependent on TXA_2 formation [14]. Unlike the peptidoleukotrienes, its action was associated with neutrophil infiltration. Reports that PGE_2 is released from isolated guinea pig lung perfused with SRS and that circulating PGI_2 and TXA_2 are increased in this species following infusion with LTC_4 complicate the issue [15, 16]. Since PGI_2 is a physiological antagonist of TXA_2, TXA_2 mediation of LTC_4 effects would be expected to be blunted by the simultaneous formation of PGI_2. From these findings it is evident that, in addition to a mediator role of TXA_2 for LT action in the guinea pig, the simultaneous formation of other cyclooxygenase-derived products can oppose this action. This may well explain the reinforcing effect of indomethacin on LTD_4 suppression of compliance following aerosol administration of the latter in the guinea pig [13]. This ability of LTs to induce the formation of cyclooxygenase-derived products appears to be due to an effect on the phospholipase that furnishes substrate AA [17].

In humans and rats, unlike guinea pigs, the physiological effects of peptidoleukotrienes do not appear to depend upon TXA_2 formation [18]. However, as with guinea pig lung, passively sensitized human lung fragments release prostaglandins when incubated with SRS [19]. Accordingly, it seems likely that prostaglandins so formed may also play a negative regulatory role on leukotriene action and/or formation in humans.

Inhibitory action of prostaglandins on leukotriene synthesis

Studies on rat peritoneal and human peripheral PMN demonstrated the ability of the chemoattractant, f-MetLeuPhe, to stimulate the release of LTB_4. PGE_1 and PGE_2 inhibited this release in a dose-related manner with an IC_{50} of 10^{-8} M for rat peritoneal and 10^{-7} M for human peripheral PMN [20]. Evidence was provided to show that PGI_2 and PGI_2-mimics have similar effects. The correlation between the inhibitory action of prostaglandins on LTB_4 release with increased intracellular cyclic AMP provided convincing evidence that this action of PGs occurs at the level of a PG receptor on the granulocyte [20]. The established ability of LTB_4 to promote sticking of PMN to the vascular endothelium [21] and the report that PGI_2 diminishes the adhesion of human PMN to endothelial cells [22] pose the possibility (Fig. 2) that the inhibitory action of PGs on LTB_4 production may be a critical factor in the emigration of the granulocyte through the blood vessel wall into the inflamed site (Fig. 2). However, a negative control on LTB_4-induced adherence of

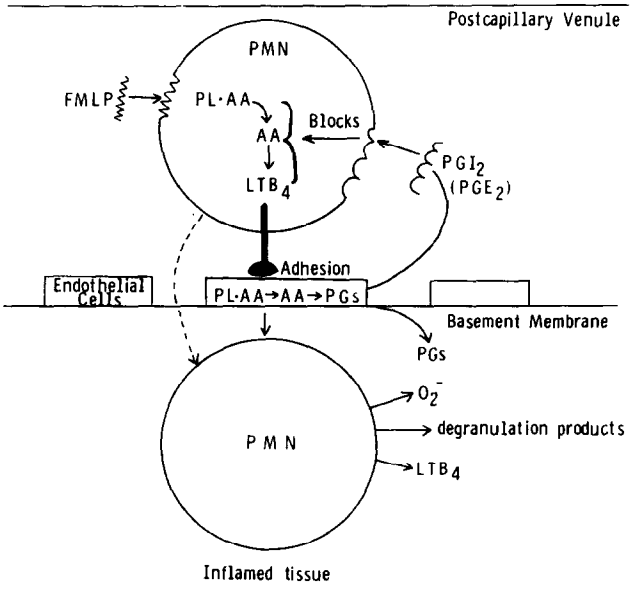

Fig. 2. Proposed interactions between PMN and endothelial cells. (From Ref. 20.)

the PMN to the endothelial cell is also possible. In either event, it is now clear that prostaglandins, by their inhibitory actions on LTB_4 formation, can have a direct effect on PMN function, in addition to their established role in enhancing blood flow in inflammatory processes.

Since the site of action of PGs in suppressing LTB_4 formation is at the initial step in the formation of leukotrienes [20], a similar inhibitory action of PGs may occur in cells associated with the production of LTC_4 and LTD_4 (S-LTs). Reports that prostaglandins inhibit the release of SRS from IgG-activated rat peritoneal leukocytes and -activated lung tissue may now be explained on the basis of inhibition by prostaglandins of the synthesis of 5-lipoxygenase-derived products [5, 23].

Effects of non-steroidal antiinflammatory agents on leukotriene production and action

Initial evidence demonstrating the ability of non-steroidal antiinflammatory agents (NSAIDs) to stimulate SRS-A release was provided by studies on human chopped lung tissue, using indomethacin [24]. Experiments with perfused sensitized guinea pig lungs revealed a similar stimulatory effect of indomethacin, aspirin and meclofenamic acid on SRS-A production [25]. In both instances, the synergistic effects of NSAIDs were suggested to be due to a reversal of an inhibitory action of PGs on SRS-A production. Based on the finding that indomethacin augments the release of SRS with a concomitant inhibition of TXB_2 formation, the stimulatory effect of indomethacin on SRS release from guinea pig lung was suggested to result from the diversion of substrate AA from the PG to the leukotriene pathway (see Fig. 1) [26, 27]. There are many reports that NSAIDs enhance or induce leukotriene-like responses, and the existence of a group of patients who respond to aspirin and other NSAIDs with symptoms of asthma emphasizes the importance of these phenomena [24]. Current dogma appears to favor the belief that NSAIDs induce such SRS responses and symptoms of asthma by shunting substrate AA from the PG to the 5-lipoxygenase pathway.

In considering the question of the shunting of AA from one pathway to the other, it is necessary to consider whether added arachidonic acid alone can result in the synthesis of leukotrienes. With respect to the conversion of AA to prostaglandins this is certainly true in many instances, a finding not surprising in view of the location of the cyclooxygenase at the endoplasmic reticulum which is proximal to plasma membrane of the cell. Despite this fact, release of AA from phospholipid pools is generally considered to be the manner by which PG synthesis is initiated and is the basis of the mechanism by which glucocorticoids inhibit PG production. Activation of cyclooxygenase, the initial enzymatic step, is not considered to be a factor in the formation of prostaglandins.

* H. W. Dougherty and F. A. Kuehl, Jr., unpublished findings.

Table 1. Products derived from endogenous ^{14}C-AA and exogenous ^{3}H-AA with RBL-1 cells

Additions	LT	PGD_2
A23187	^{14}C	—
^{3}H-AA	—	^{3}H
A23187 + ^{3}H-AA	^{14}C	^{3}H

The cytosolic nature of 5-lipoxygenase, the initial enzymatic step leading to leukotrienes, does not foster the concept that exogenously added arachidonic acid alone would necessarily stimulate leukotriene production. Such an action would require circumvention of triglycerides and phospholipid forming enzymes present throughout the cell for the accumulation of free AA within the cytosol in quantities sufficient to result in the formation of leukotrienes. In studies with the human PMN, added [^{3}H]arachidonic acid was shown to effect little incorporation of tritium into LTB_4 unless a stimulator such as ionophore A-23187 was present [28]. Although these workers interpreted this to mean that activation of the 5-lipoxygenase by ionophore is required for utilization of externally added arachidonic acid, the alternate possibility, the requisite prior incorporation of AA into a phospholipid pool responsive to ionophore, could also explain these data. Studies in our laboratories favor this latter concept.

In such studies, RBL-1 cells were prelabeled in their phospholipid pools by preincubation with [^{14}C]AA*. As shown in Table 1, treatment of these cells with ionophore A23187 resulted in the synthesis of ^{14}C-leukotrienes alone. Addition of [^{3}H]AA promoted the synthesis of [^{3}H]PGD_2 exclusively, but no leukotrienes were formed. Exposure of cells, prelabeled with [^{14}C]AA, to [^{3}H]AA in the presence of A23187 for a short period resulted in the expected release of [^{3}H]PGD_2 but only ^{14}C-leukotrienes were detected. This failure to yield ^{3}H-leukotrienes, even in the presence of ionophore, implies that activation of 5-lipoxygenase is not a critical factor in leukotriene formation. Alternatively, it appears that prior incorporation in phospholipid pools is an obligatory requirement for formation. Such an interpretation is in accord with the expectation that the inhibitory action of steroids on leukotriene production is at the phospholipase, as has been established for their effects on PG production.

NSAID action on cells which synthesize both prostaglandin and leukotriene

The mouse peritoneal macrophage has been shown to produce large amounts of PGE_2 and PGI_2 (as 6-K-$PGF_{1\alpha}$) as well as LTC_4 and LTB_4 in response to zymosan [29, 30]. Inhibition of cyclooxygenase by indomethacin caused no significant increase in LTC_4 release despite a significant rise in available substrate arachidonic acid. The recent report that zymosan stimulates the release of both PGE_2 and LTC_4, whereas the membrane stimulator phorbol myristate acetate (PMA) affects an increase in PG formation alone, implied the presence of separate pools of AA

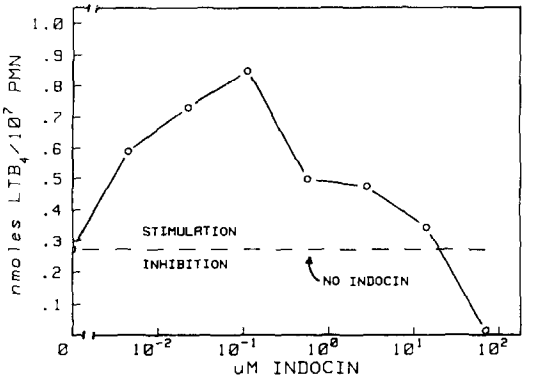

Fig. 3. Indocin effect on LTB_4 formation by rat peritoneal PMN.

for each metabolic pathway [31]. This interpretation was strengthened by the observation that the effects of zymosan and PMA on the macrophage are additive with respect to PG formation*. Thus, individual pools of substrate AA exist for LT and PG productions and excessive free AA formation as a consequence of NSAID action does not result in a significantly increased conversion of AA to leukotrienes.

The RBL-1 cell, like the macrophage, also has a large biosynthetic capacity for prostaglandin and leukotriene formation. As with the macrophage, the suppression of prostaglandin formation by indomethacin or aspirin in A23187-stimulated RBL-1 cells did not result in an enhancement of leukotriene synthesis [32].

Oxygenation of AA by the PMN proceeds largely by the lipoxygenase pathway; relatively little cyclooxygenase-derived products are formed [33].

Studies in the rat peritoneal PMN revealed that extremely low doses of indomethacin caused a marked enhancement of LTB_4 (Fig. 3). As shown, this effect is dose related until high non-physiological levels of indomethacin are used†. The transformation from stimulation to inhibition of LTB_4 by high levels of indomethacin is believed to be attributable either to direct inhibition of 5-lipoxygenase or to the known effect of this NSAID to compete for the formyl methionyl leucyl phenylalanine (FMLP) receptor [34]. Suppression of PGE_2 synthesis by indomethacin was confirmed by direct measurement of PGE_2 formed wherein indomethacin diminished a control level of 7 ng PGE_2 per 10^7 PMN per 2 ml in a dose related manner*. This concentration of PGE_2 (10 nM) is comparable to the IC_{50} for inhibition of LTB_4 synthesis by PGE_2 (5 nM) and would be expected to significantly suppress LTB_4 production.

Intercellular transfer of substrate arachidonic acid

Stimulation of aspirin-treated human platelets, prelabeled in their phospholipid pools with [^{3}H]AA, with ionophore A23187 in the presence of PMN has been shown to result in a synthesis of [^{3}H]LTB_4 by the latter. Since platelets are unable to synthesize LTB_4, it is clear that they are capable of furnishing substrate AA for metabolism by the PMN [35]. The biological significance of these findings could be questioned since the PMN must be activated for utilization of AA by the 5-lipoxygenase pathway. However, the report that (12*S*-12-hydroperoxy-5,8,10,14-icosatetraenoic acid (12-HPETE) is capable of affecting the release of LTB_4 and the derivation of 12-HPETE in quantity from the platelet does provide persuasive evidence that such a transfer of substrate AA can occur under conditions in which the platelet alone is activated and, of course, inhibition of platelet cyclooxygenase activity could result in such a transfer of AA to the PMN [36].

In summation, it is clear that interactions between leukotrienes and prostaglandins can occur at many levels (Fig. 4). In cells where both pathways prevail,

* J. L. Humes, personal communication.

† E. A. Ham, D. D. Soderman and F. A. Kuehl, Jr., unpublished findings.

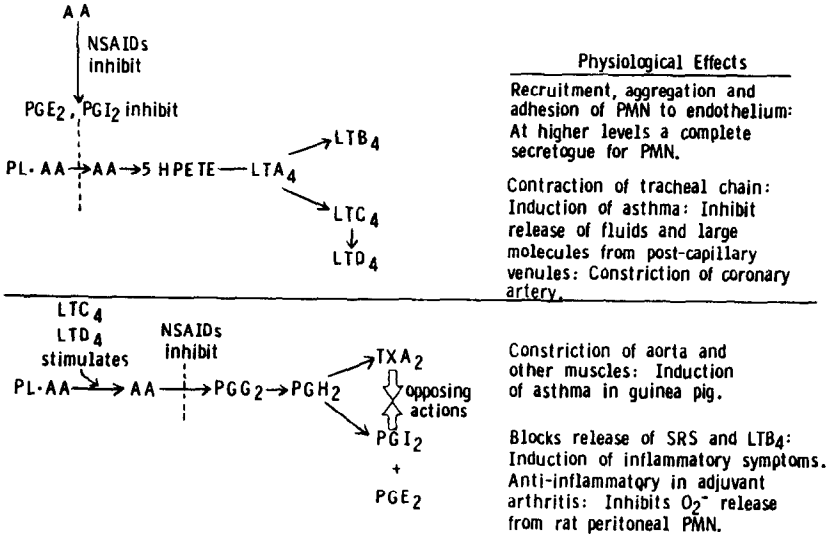

Fig. 4. Interactions between leukotrienes and prostaglandins.

substrate AA appears to be derived from different pools. On the other hand, intercellular transfer of substrate AA can occur. AA released from the platelet can trigger LTB_4 production by the PMN providing the latter cell is activated. TXA_2, a product of the PG pathway, can mediate actions of peptidoleukotrienes in the guinea pig. On the other hand, the ability of leukotrienes to also stimulate PGI_2 which can act in opposition to LTC_4 and LTD_4 assigns a modulating role for PGs in leukotriene action. Finally, the recent report that prostaglandins can regulate the synthesis of leukotrienes adds an important new interrelationship between these two classes of cell regulators that may be the basis for the action of NSAIDs in inducing LT release and symptoms of asthma.

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