

# Editorial

## Leukotrienes and lung disease

Leukotrienes, which are derived from arachidonic acid and have similar chemical structures to the prostaglandins, have recently been shown to contain most of the biological activity previously attributed to "slow reacting substance of anaphylaxis" (SRS-A). The term "slow reacting substance" was first used by Feldberg and Kellaway<sup>1</sup> to describe an activity obtained from guinea pig or cat lung perfused with cobra venom and which gave a slow sustained contraction of the guinea pig ileum. An agent with similar pharmacological properties was identified from perfusates of sensitised guinea pig lung challenged with specific antigen and was designated SRS-A to distinguish it from other SRSs released non-immunologically.<sup>2</sup> For many years SRS-A has been considered to be a pharmacological mediator of particular importance in the pathogenesis of bronchial asthma and possibly other forms of airways disease, since it (a) gave a prolonged sustained contraction of human bronchial smooth muscle preparations, (b) had high biological activity at chemically undetectable concentrations, (c) produced bronchospasm when inhaled by asthmatic volunteers,<sup>3</sup> and (d) was released from the lung of asthmatic subjects (obtained at operation) after incubation with the allergen to which they were known to be hypersensitive.<sup>2,4</sup> Nevertheless, the precise role of SRS-A in bronchial asthma and related diseases was unlikely to become apparent until the agent had been fully characterised chemically. Although the exact covalent structure and stereo-chemistry of the SRS-A leukotrienes are known and most of them are available in synthetic form, their role in the pathogenesis of asthma is still unclear, but these important advances in our understanding of SRS-A prompt a reappraisal of their possible significance in the various forms of obstructive airways disease.

### Chemistry

An account of the detailed chemistry of the leukotrienes and their discovery can be found elsewhere.<sup>5</sup> Leukotrienes are a family of closely related hydroxy

fatty acids derived from arachidonic acid (eicosatetraenoic acid, ETE).<sup>\*</sup> Arachidonic acid is contained in the membrane phospholipids of most mammalian cells, including those of the mast cell/basophil series, and can be released by a number of stimuli including the interaction of IgE with specific allergen. Cyclo-oxygenase enzymes convert arachidonic acid to the prostaglandins, thromboxanes and prostacyclin, whereas the various lipoxygenases form the hydroperoxy eicosatetraenoic acids (HPETEs). These are further metabolised to hydroxyeicosatetraenoic acids (HETEs) and leukotrienes (fig 1). The leukotrienes (LT) were named because of their proposed leucocyte origin and since three of their four double bonds are present as a conjugated triene.<sup>\*</sup> The subscript "4" refers to leukotrienes with four double bonds—that is, LTB<sub>4</sub>, LTC<sub>4</sub> and so on.

In the past two years or so investigators in Sweden, the United States, and the United Kingdom have established that the activity which comprises SRS-A is almost entirely attributable to leukotrienes C<sub>4</sub>, D<sub>4</sub>, and E<sub>4</sub> (LTC<sub>4</sub>, LTD<sub>4</sub>, and LTE<sub>4</sub>). These compounds are derived from 5-HPETE via the unstable epoxide intermediate, leukotriene A<sub>4</sub> (LTA<sub>4</sub>) (fig 1). Leukotriene C<sub>4</sub> is a 20 carbon hydroxy fatty acid linked to the tripeptide, glutathione. Peptidyl transferases present in most body fluids cleave the terminal amino-acids from LTC<sub>4</sub> to yield LTD<sub>4</sub> and LTE<sub>4</sub>. For instance, it has been shown that radio-labelled LTC<sub>4</sub> is rapidly converted in vivo to LTD<sub>4</sub> and LTE<sub>4</sub>.<sup>6</sup> LTB<sub>4</sub> is a 5,12 dihydroxy derivative of arachidonic acid and has several naturally occurring isomers. It possesses relatively little SRS-A-like activity but it is a potent chemoattractant—that is, it promotes migration of leucocytes both in vivo and in vitro.<sup>7</sup>

### Sources of leukotrienes

It is probable that mast cells, including human lung mast cells, are a major source of leukotrienes as are basophils, neutrophils, and macrophages. It has been shown in experimental animals that, in certain types of inflammatory reaction, a specialised subset of macrophages produce particularly large amounts of leukotrienes and other lipoxygenase products.<sup>8</sup>

<sup>\*</sup>See Glossary.

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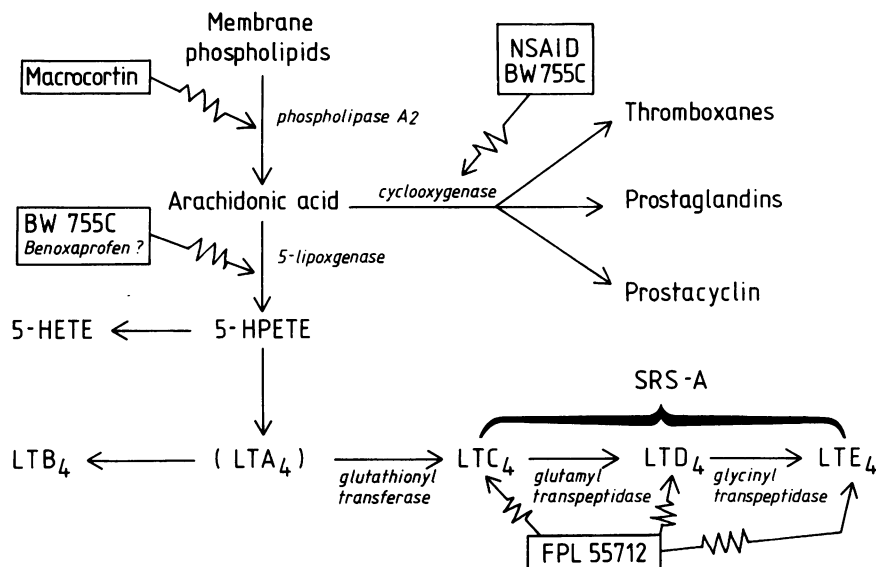


Fig 1 Pathways of arachidonic acid metabolism and the sites of action of various inhibitors and antagonists.

### Release mechanisms

Since leukotrienes are derived from membrane phospholipids they can be considered as newly-formed mediators, in contrast to histamine which exists in a preformed state in mast cell granules. It is now recognised that a number of stimuli, both immunological and non-immunological, lead to the generation by various leucocytes of leukotrienes and other lipoxygenase products. Such stimuli include phagocytosis, treatment with agents which increase intracellular calcium (that is, the Ca ionophore) as well as the interaction of specific antigen with cell-bound IgE. Therefore, lipoxygenase products are a feature of inflammatory reactions in general and are not solely confined to "allergic", that is, IgE-mediated responses. A number of membrane events precede the availability of free arachidonic acid which follows the appropriate stimuli. These include disorientation and translocation of membrane phospholipids, influx of calcium ions and activation of phospholipase A<sub>2</sub>, the enzyme which hydrolyses membrane phospholipids and releases free arachidonic acid for use in the synthesis of leukotrienes (fig 2).

### Biological activities

In general, leukotrienes with SRS-A-like activity (LTC<sub>4</sub>, LTD<sub>4</sub>, and LTE<sub>4</sub>) contract smooth muscle

whereas LTB<sub>4</sub> and the HETEs are chemotactic—that is, they give rise to an infiltration of inflammatory cells. Various HETEs also stimulate mucous gland secretion.<sup>9</sup>

LTC<sub>4</sub> and LTD<sub>4</sub> produce an appreciable contraction of human bronchial smooth muscle preparations in vitro at concentrations as low as 10<sup>-8</sup> mol.l<sup>-1</sup>.<sup>10</sup> In this respect they are approximately 1000 times more potent than histamine. Smooth muscle from human pulmonary artery and vein also contracted in response to LTC<sub>4</sub> and LTD<sub>4</sub>.<sup>10</sup> There is also evidence that leukotrienes decrease the rate of mucus clearance from asthmatic airways after antigen inhalation.<sup>11</sup>

The effects of the leukotrienes on the micro-vasculature are complex. In the guinea pig LTD<sub>4</sub> dilates, whereas LTC<sub>4</sub> constricts the small blood vessels of the skin.<sup>12</sup> In man both of these leukotrienes evoke a wheal and flare reaction when injected intradermally. After intravenous injection to guinea pigs, leukotrienes C<sub>4</sub> and D<sub>4</sub> induce some of the features of systemic anaphylaxis such as arterial hypotension,<sup>12</sup> impaired left ventricular performance,<sup>13</sup> and reduced coronary arterial blood flow.<sup>13</sup> Several lipoxygenase products have been shown to increase the amount of mucus glycoprotein synthesis by human airways tissue indicating that this might contribute to the mucus plugging characteristic of bronchial asthma.<sup>9</sup> Not only are leukotrienes potent mediators per se but they also interact synergistically with other agents such as prosta-

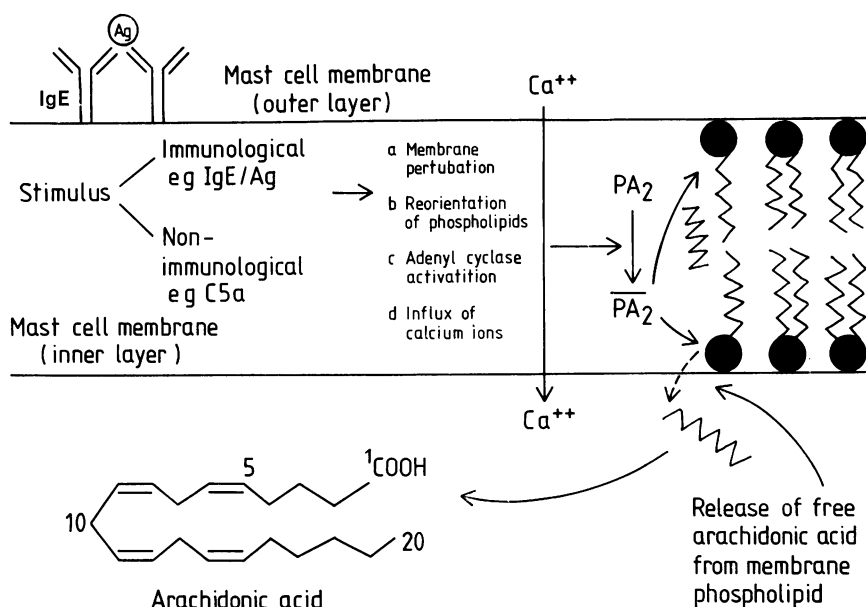


Fig 2 A diagrammatic representation of arachidonic acid formation from mast cell membrane phospholipids. After the combination of specific antigen (Ag) with immunoglobulin (IgE), calcium enters the cells and arachidonic acid is released from membrane phospholipids as the result of activation of phospholipase  $\text{A}_2$  ( $\text{PA}_2$ ).

glandins and the complement derived anaphylatoxin,  $\text{C5a}$ .<sup>14</sup>

#### BRONCHIAL ASTHMA

Leukotrienes and HETEs have the potential for producing many of the pathophysiological features of asthma, for example bronchospasm, mucosal oedema, cellular infiltration, mucus production, and impaired mucus clearance. Their effects on the microvasculature may contribute to ventilation-perfusion imbalances. SRS-A-like activity has been demonstrated in the sputum of asthmatic patients,<sup>15</sup> although it has yet to be shown that this activity was caused by  $\text{LTC}_4$  and  $\text{LTD}_4$ . Recent work from this laboratory has identified  $\text{LTB}_4$  metabolites in asthmatic sputum (unpublished observation). Nevertheless the role of SRS-A in asthma still remains unproven and is likely to remain so until specific leukotriene antagonists have been shown to be efficacious in the treatment of the disease. In this regard it is of particular interest that the inhalation of synthetic leukotrienes by normal human volunteers caused bronchoconstriction as well as coughing; both were partially inhibited by specific leukotriene antagonists FPL 55712 and FPL 59257.<sup>16</sup>

It is probable that both histamine and leukotrienes  $\text{C}_4$  and  $\text{D}_4$  are involved in the bronchoconstriction that follows inhalation of antigen by

asthmatic subjects. Passively sensitised human lung tissue when challenged with antigen in a perfused organ bath, gave an initial contraction which was inhibited by an antihistamine,<sup>17</sup> but the contraction also had a sustained (4-15 min) phase which was inhibited by FPL 55712. This suggests that the initial phase of antigen-induced bronchoconstriction is mediated by histamine whereas the prolonged phase is mediated by SRS-A.

Although lipoxygenase products have potential to contribute to the disease process in asthma, considerably more information is required before their true importance is known.

#### CHRONIC BRONCHITIS

This disease also involves increased mucus production, airways obstruction, and the presence of inflammatory cells in the sputum, situations in which the leukotrienes and HETEs might be associated. Turnbull *et al* described the presence of histamine, SRS-A-like activity and IgE in the sputum of certain patients with chronic bronchitis.<sup>15</sup> However, the nature of this SRS-A-like activity and its relation to lipoxygenase products remain to be established.

#### CYSTIC FIBROSIS (CF)

Cromwell *et al* have identified leukotrienes  $\text{B}_4$ ,  $\text{C}_4$ ,

and D<sub>4</sub> in the sputum of patients with this disease.<sup>18</sup> These mediators might contribute to the substantial degree of airways obstruction, increased sputum production and sputum neutrophilia which are characteristic of CF. The origin of these lipid mediators in the sputum is unknown but their production could be related to atopy, a common feature in CF patients.

### Modulation by drugs

A number of compounds are currently available which either inhibit leukotriene and HETE production or antagonise their end organ response. At the present time only a few are suitable for clinical studies.

#### SRS-A ANTAGONISTS

FPL 55712 is a specific antagonist of LTC<sub>4</sub> and LTD<sub>4</sub>, probably acting by competitive antagonism. It is not absorbed orally and has a very short half-life. A small open trial showed a minimal degree of improvement when FPL 55712 was administered by aerosol to patients with severe asthma.<sup>19</sup> A new Fisons product, FPL 59257, is orally active and has a longer pharmacological half-life.<sup>16</sup>

#### LIPOXYGENASE INHIBITORS

Benoxaprofen is a non-steroidal anti-inflammatory drug which is currently in use as an anti-arthritis agent. In certain animal models it is a potent inhibitor of lipoxygenase enzymes, but has relatively weak effects on cyclooxygenase pathways. It is currently being evaluated for its role in human lung disease. BW 755C is an experimental drug which inhibits both the lipoxygenase and the cyclooxygenase pathways, thus preventing the synthesis of both prostaglandins and leukotrienes.

#### CORTICOSTEROIDS

These agents exert their anti-inflammatory effect by various mechanisms, one of which involves the generation of newly synthesised intracellular proteins which function as "second messengers".<sup>20</sup> These proteins, which include macrocortin and lipomodulin, inhibit the action of phospholipase A<sub>2</sub>, so preventing the release of free arachidonic acid and subsequent generation of leukotrienes and prostaglandins (fig 1).

#### CALCIUM ANTAGONISTS

Drugs such as verapamil or nifedipine partially inhibit exercise-induced bronchospasm.<sup>21</sup> They may act by reducing smooth muscle responsiveness, but in high concentrations verapamil also inhibits SRS-A release.<sup>22</sup> Disodium cromoglycate also inhibits

calcium flux in certain in vitro systems and part of its mode of action in asthma might be explained by its ability to indirectly prevent arachidonic acid metabolism. This view is supported by in vitro experiments in which cromoglycate was shown to reduce the amount of SRS-A released by sensitised human lung fragments after exposure to specific antigen.<sup>23</sup>

### Concluding comments

It seems likely that the leukotrienes play a major aetiological role in the pathogenesis of asthma and related diseases although the true extent of their involvement in the events leading to airways narrowing is still unknown. Further studies are required to clarify their precise modes of action. The identification of leukotrienes in the blood and other body fluids of asthmatic subjects, using sensitive and reproducible assays, will enhance considerably our knowledge of their participation in this disease. Finally, the possibility that non-toxic drugs might be available which either prevent the formation of leukotrienes or selectively inhibit their mode of action holds considerable promise for the treatment of asthma.

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### Glossary

Arachidonic acid is eicosa tetraenoic acid; "eicosa" describes it as a 20 carbon fatty acid; "tetraenoic" describes its four double bonds.

A conjugated triene is a section of the carbon "backbone" of a fatty acid, where three double bonds occur at alternate carbon atoms, for example, in the triene section of LTD<sub>4</sub>, the double bonds occur at carbon atoms 7, 9, and 11. The triene structure produces a characteristic ultraviolet absorption spectrum.

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