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L. WALCH, X. NOREL, J.P. GASCARD, C. BRINK

CYSTEINYL-LEUKOTRIENE RECEPTORS IN PULMONARY VESSELS

CNRS ESA 8078 133 av. de Résistance 92350 LePlessis Robinson, France.

Two categories of cysteinyl-leukotrienes have been proposed, namely, $CysLT_1$ and $CysLT_2$. These receptors are found not only on the vascular smooth muscle but also on the endothelium. Activation of the receptor(s) on vascular smooth muscle provokes contraction whereas activation of the receptors on the endothelium produces contraction and/or relaxation. These endothelium dependent effects are due to the release of both contractile and relaxant factors derived from the endothelium. While factors derived from either the cyclooxygenase or nitric oxide pathways are involved, in some vascular preparations other mediators such as endothelin may be involved. However, in isolated human pulmonary vascular preparations, this appears not to be the case and presently the nature and origin of the contractile factor remains to be established.

K e y w o r d s: *leukotrienes, anaphylaxis, vascular smooth muscle, asthma, leukotriene receptors, endothelium.*

INTRODUCTION

Dyspnea and severe hypotension have been reported to be the cardinal signs of severe anaphylactic reactions in both animals and man. Milder allergic inflammatory responses are frequently associated with an increased blood flow, extravasation of plasma and the recruitment of circulating leukocytes into the tissue compartment. Thus a cardinal sign of activation of inflammatory cells by allergen is a marked alteration in vascular tone and reactivity.

The production of slow reacting substance of anaphylaxis (SRS-A) was always associated with antigenic stimulation of sensitized tissues. Schild and coworkers (1) demonstrated that human lung from asthmatic patients released SRS-A when stimulated with an appropriate antigen. This observation was confirmed by the work of Brocklehurst (2) and Dahlén and coworkers (3). Other investigators have shown that human lung tissues passively sensitized

(4, 5) and then challenged with antigen also released these potent mediators. Since the original description of SRS-A, this entity is now known (6) to be a composite of leukotrienes (LTC_4, LTD_4 and LTE_4) which are metabolites of the arachidonic acid cascade *via* the 5-lipoxygenase pathway. The cysteinyl-leukotrienes (cysLTs) are known to be potent contractile and relaxant agents in the pulmonary vascular bed. These mediators are also known to induce the release of both contractile and relaxant factors.

The aim of the present report is to provide some recent cysLT data obtained from the human lung and to highlight the effects of cysLT in pulmonary vascular tissues.

MATERIALS AND METHODS

Isolated human vascular preparations were obtained from five patients undergoing surgery for cancer. Pulmonary arteries and veins were cut as rings and equilibrated in Tyrode's solution using the methods which have previously been published (7). Subsequent to an equilibration period and after washing in fresh Tyrode's solution the tissues were challenged with noradrenaline (10 μM) and after 15 min leukotriene D_4 (LTD_4; 10 μM) was added to the organ baths. After 10 min the bath fluid was collected and stored at −20 °C until analysis.

Endothelin-1 was assayed using an enzymoimmunological assay and the measurements were performed according to the directions in the kit (SpiBio, Cayman, France).

RESULTS

The results presented in *Table 1* indicate that the levels of endothelin-1 were below the threshhold of detection of the kits. These experiments were performed under conditions where LTD_4 has been reported to release a contractile factor from vascular preparations of the human lung.

Table 1. Production of Endothelin-1 by Isolated Human Pulmonary Vascular Tissues after challenge with LTD_4.

Preparations	Wet weight (mg)	Endothelin-1
Pulmonary artery	306±21	Not detected
Pulmonary vein	286±9	Not detected

Values are means±SEM from 5 patients. Tissues were challenged with noradrenaline (10 μM) and after 15 min LTD_4 (10 μM) was added.

DISCUSSION

The release of SRS-A from the aorta of sensitized guinea-pigs during antigen challenge has been reported by Brocklehurst (2). Piper and coworkers (8) detected the release of leukotrienes from the porcine pulmonary artery during ionophore stimulation and these investigators also showed a release from human pulmonary arteries when stimulated with anti-IgE. Recently Gorenne and coworkers (7) confirmed and extended these results by demonstrating that the release of cysteinyl-leukotrienes (cysLTs) induced by antigen was also observed in human pulmonary veins and in both types of preparations (arteries and veins) the release was modified by inhibition of the cyclooxygenase pathway since the quantities of cysteinyl-leukotrienes released were increased in tissues treated with indomethacin. These results suggest that the local production of cyclooxygenase metabolites regulated the amounts of cysteiny-leukotrienes released.

There is a considerable amount of evidence which demonstrate that antigen contracts pulmonary arteries obtained from bovine and guinea pig (9—12). These antigen-induced contractions were enhanced in the presence of indomethacin and not blocked by FPL 55731. These investigators concluded that either metabolites of the cyclooxygenase pathway were responsible for offsetting the contraction or products of the lipoxygenase pathway were preferentially activated when the cyclooxygenase pathway was inhibited and this enhanced production of 5-LO metabolites led to the increased response observed during antigen challenge. Kelly and coworkers (13) confirmed these observations and demonstrated that in airways and pulmonary arteries from the guinea pig the ***CystLT*** $_1$ antagonist (SKF104353) abolished the residual contraction observed after histamine receptor blockade. However, these investigators observed a striking difference between the kinetics of contraction. The arterial preparations exhibited an immediate but unsustained contraction to antigen stimulation whereas the airways showed a more protracted and sustained response. One possible explanation for this kinetic difference may be that a functional antagonism exists due to the release of relaxing factors derived from the endothelium. However, no attempt was made by these authors to explore either the receptors involved or the mechanisms associated with these effects. In contrast, isolated human pulmonary arteries do not contract when challenged with antigen (14). However, these preparations relaxed when challenged with antigen under conditions of elevated tone. These relaxations were also observed when contracted tissues were challenged with cysLTs. Thus the antigen effects are mimicked by the cysLTs. In addition, a contractile factor is released from the endothelium subsequent to either antigen or cysLT stimulation however, this mediator appears not to be endothelin (present report).

Two categories of receptors for the cysteinyl-leukotrienes have been proposed (15). One subtype is characterized by the ability of a number of antagonists to block the effects of cysteinyl-leukotrienes in a variety of smooth muscle preparations. This receptor is referred to ***CysLT*** $_1$. The effects associated with activation of the second receptor (***CysLT*** $_2$) are not blocked by these antagonists. In vascular preparations, Nishiye and co-workers (16) showed that FPL55712 and ONO-RS−411 blocked the LTD_4 contractions in the guinea pig basilar artery implicating activation of a ***CysLT*** $_1$ receptor. The use of a number of selective ***CysLT*** $_1$ antagonists demonstrated that the contractions induced by cysLTs in human pulmonary veins were not affected by these antagonists (17). This receptor on the human pulmonary veins is therefore a ***CysLT*** $_2$. In contrast, Rinkema and co-workers (18) demonstrated that the LTD_4 contractions in the guinea pig inferior vena cava were blocked by LY171883 and WY48252 (***CysLT*** $_1$ antagonists). However, the contractions induced by LTC_4 were blocked in a biphasic fashion by these two ***CysLT*** $_1$ antagonists, that is, the low concentrations of LTC_4 were not affected by the antagonists suggesting two LTC_4 receptor subtypes. Therefore, in some species, such as the guinea pig, vascular preparations may contain either one or several subtypes of cysteinyl-leukotriene receptors. Whether these receptors in the human pulmonary veins which are resistant to the classical antagonists are the same as the receptors in the guinea pig pulmonary artery which are activated by the low concentrations of LTC_4 remains to be established.

On the endothelium of guinea pig arterial preparations, a single receptor is present (19, 20) and is associated with relaxation. This is not the case in either the canine renal arteries and veins (21) or in the human pulmonary arteries and veins (22). In canine preparations the renal veins relaxed to LTD_4 but veins were approximately 100-fold more sensitive to this mediator when compared with the arteries. This difference in agonist potency suggests that different receptors may be present on the endothelium of these vascular preparations. LTC_4 was not examined in these tissues. In canine splanchnic venous capacitance vessels the receptors associated with the relaxations induced by cysLTs have not been identified.

The endothelium in human pulmonary arteries has one receptor (***CysLT*** $_2$) and activation induced the release of nitric oxide (NO). However, in isolated human pulmonary veins two receptors are present, a ***CysLT*** $_1$ and ***CysLT*** $_2$. Activation of the former induced the release of a contractile factor whereas activation of the ***CysLT*** $_2$ receptor released NO. The contractile factor appears not to be endothelin-1 since this agents was not detectable in these preparations. In guinea pig pulmonary artery and guinea pig thoracic aorta, one receptor has been demonstrated since the relaxations are blocked by ICI198615. These data suggest the presence of a ***CysLT*** $_1$ receptor. Activation of this receptor leads to the release of a relaxant factor, namely, NO. In contrast,

in human pulmonary arteries and veins activation of a receptor which is resistant to ICI198615 is associated with NO release. These results suggest that there may be species differences even when analogous vascular preparations are examined

While the cysLTs are known to relax vascular smooth muscle in a variety of preparations from different species, there are presently two pathways involved in this response. One involves the metabolites of arachidonic acid *via* the cyclooxygenase enzymatic pathway and the other implicates products of the L-arginine enzymatic pathway. Although both pathways may be present and active in the endothelium of the vascular preparations only one of these enzymes may be dominant and be responsible for the relaxations observed. Ortiz and co-workers (23) have demonstrated that in pulmonary veins the dominant pathway for cysLTs relaxations is the NO pathway. There are some reports from animal studies which support a dominant role for NO in pulmonary veins (24—26). In contrast, Allen and co-workers (27) demonstrated that the LTC_4 induced relaxations in isolated human saphenous veins were not modified by treatment of tissues with an NO inhibitor but were significantly enhanced following treatment with indomethacin. These authors suggested that a contracting factor derived from the arachidonic acid pathway was released in preparations challenged with LTC_4. In addition these investigators demonstrated that the NO inhibitor had no effect on the LTC_4 relaxations. Together, these results suggest that cysLTs effects in human pulmonary veins are dominated by the NO pathway whereas in human systemic veins these mediator effects are modified by metabolites of the cyclooxygenase pathway. A summary (*Schema I*) provides an outline of the information concerning the ***CysLT*** receptors and indicates that only veins when activated release a contractile factor. The cysLTs may have a prominent role in the activation of the cyclooxygenase and/or NO pathways and further studies of the receptors involved may help to elucidate their modulatory role in vascular tone and reactivity.

Schema I
CysLT receptors on the endothelium.

Preparations	Receptors	Factors Released
Guinea pig pul. artery	$CysLT_1$	Nitric oxide
Human pul. artery	$CysLT_2$	Nitric oxide
Human pul. vein	$CysLT_1$ $CysLT_2$	Contractile factor Nitric oxide
Human saph. vein	?	Contractile factor Nitric oxide

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Author's address: Charles Brink PhD, Centre Chirurgical Marie Lannelongue CNRS ESA 8078, 133 av. de la Résistance, 92350 Le Plessis Robinson, tel: 33.1.40.94.28.00 ext 3015, Fax: 33.1.46.30.12.08, Email: brink@pratique.fr, CNRS ESA 8078, 133 av. de la Résistance, 92350 LePlessis Robinson, France