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Commentary

Cysteinyl leukotriene receptors

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Abstract

Cysteinyl leukotrienes (CysLTs) are important inflammatory mediators in asthma and allergic disorders. Two types of CysLT receptors, $CysLT_1$ and $CysLT_2$, which were originally defined pharmacologically based on their sensitivity to $CysLT_1$ specific antagonists, are responsible for most of the known CysLT biological actions. The regulation of CysLT receptor expression and signaling in disease processes is largely unclear. Recent molecular cloning of both receptor subtypes from several different species will greatly facilitate future research in understanding CysLT signal transduction mechanisms. Expression of the relatively better-studied $CysLT_1$ is verified in lung tissues and peripheral blood cells. Elucidating how this receptor mediates airway inflammation will deepen our understanding of asthma etiology. On the other hand, detection of $CysLT_2$ in the heart, brain, and adrenal glands will inject new excitement into the search for novel CysLT functions. This review summarizes receptor cloning, ligand binding, expression, signaling, and functions in an effort to bridge early pharmacological studies to future studies at the molecular level.

Keywords: Cysteinyl leukotriene; Receptors; Cloning; GPCR; Expression; Asthma; Function

1. Introduction

The study of the CysLTs LTC_4, LTD_4, and LTE_4, known historically as slow-reacting substance of anaphylaxis (SRS-A), has been ongoing for more than twenty years [1]. CysLTs are important inflammatory mediators that play a major role in the pathophysiology of inflammatory diseases such as asthma and allergic rhinitis.

CysLTs exert their effects through cell surface receptors. Great efforts have been put into the characterization of the receptors ever since the discovery of these substances. In fact, FPL-55712 was identified as a specific antagonist even before the chemical components of SRS-A were elucidated [2]. Significant confusion in receptor classification existed prior to the standardization of the nomenclature in 1995 by the International Union of Pharmacologists (IUPHAR) [3]. CysLT receptors were classified into two major subtypes: $CysLT_1$, which could be blocked by a family of specific antagonists, and $CysLT_2$, which was basically insensitive to $CysLT_1$ antagonists. This classification, however, did not rule out the possibility of subdivision. Recently, the molecular cloning of both $CysLT_1$ [4–7] and $CysLT_2$ [8–11] has opened new avenues for CysLT exploration.

2. CysLT receptor cDNA and gene cloning

Numerous quests at conventional receptor purification or expression cloning proved futile for cloning CysLT receptors. Their ultimate cloning resulted from blooming sequence databases, computational mining, and high throughput screening methodologies. $hCysLT_1$ cDNA encodes a protein of 337 amino acids (aa). $mCysLT_1$ is encoded by two transcripts: a short splice variant encoding a protein of 339 aa, which aligns well with $hCysLT_1$; and a long variant encoding a protein extending 13 aa at the N terminus. The homology between $hCysLT_1$ and $mCysLT_1$ is approximately 87% at the protein level.

The open reading frame of $hCysLT_2$ encodes a protein of 347 aa, while both $rCysLT_2$ and $mCysLT_2$ deduced structures have 309 aa, truncated by 16 and 21 residues at the N- and C-termini, respectively. Homology between human and rat, and human and mouse $CysLT_2$ is 73 and 65%,

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Abbreviations: CysLTs, cysteinyl leukotrienes; $CysLT_1$ and $CysLT_2$, cysteinyl leukotriene receptor subtype 1 and subtype 2, respectively; hCysLT, mCysLT and rCysLT, human, mouse and rat cysteinyl leukotriene, respectively; GPCR, G protein-coupled receptor; IFN, interferon; IL, interleukin; PKC, protein kinase C; RT–PCR, reverse transcription–polymerase chain reaction.

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respectively. In contrast, the identity of the subtypes $hCysLT_1$ and $hCysLT_2$ is only 38%. The structures of both *mCysLT1* and *mCysLT2* genes have been characterized. The *mCysLT1* gene spans about 24 kb with 4 exons and 3 introns. Alternative splicing yields a short transcript containing exons 1 and 4 that encodes the short $CysLT_1$ variant with initiator ATG codon in exon 4 or a long transcript containing all 4 exons with initiator ATG in exon 3. $hCysLT_1$ cDNA does not have an upstream translation start site and is unlikely to have a similar long transcript. The *mCysLT2* gene is about 20 kb long with 6 exons and 5 introns. Alternative splicing of exon 3, part of the 5′-UTR, results in two transcripts with no difference in protein structure. Basically, the complete coding regions of *CysLT1* and *CysLT2* are located in the last exon, uninterrupted by introns, like most GPCR genes [12].

3. Protein structure and ligand binding of CysLT receptors

CysLT receptors have long been recognized as GPCRs prior to their cloning [13], based on the fact that binding of the ligands to the receptors is enhanced by divalent cations but inhibited by sodium ions and non-hydrolyzable GTP analogs.

Hydrophobicity analysis of the deduced primary structures reveals that both $CysLT_1$ and $CysLT_2$ have seven hydrophobic transmembrane (TM) domains linked by six hydrophilic loops, typical of GPCRs. There is no binding model currently available for leukotrienes. Based on the ligand structure, eicosanoid receptors have been classified into a subfamily of rhodopsin-/β2 adrenergic receptor-like receptors. It is highly possible that CysLTs emulate prostanoids to bind their receptors through TM core domains, especially TM3, TM6, and TM7 [14,15]. CysLT receptors also bear some homology to the purinoreceptor P2Y family, and UDP indeed appears to be an agonist at $CysLT_1$ [16]. TM3, 6, and 7 of P2Y receptors are thought to be crucial for nucleotide binding [17] as well. The homology between $CysLT_1$ and $CysLT_2$ is high in TM3 and TM7, while comparatively low in TM4–6. This sequence difference may result in the difference in ligand binding specificity between the receptors. Some critical amino acids may account for the differences in binding affinity for LTC_4 or LTD_4. Moro *et al.* [18] suggested that the extracellular loops 2 and 3, and two disulfide bridges are important in ligand contact and receptor structure stability for the P2Y1 receptor. Whether this also applies to the CysLT receptors is yet to be investigated.

Receptor binding affinity between species is similar. The $mCysLT_1$ long isoform, which has a 13 aa extension at the N terminus, shows no difference in ligand binding from either the short isoform or $hCysLT_1$ [6]; and there is no difference in binding between $mCysLT_2$ and $hCysLT_2$, even though $mCysLT_2$ is shorter by 16 aa at the N terminus [11].

4. Signaling through CysLT receptors

In each of the reported cloning studies, intracellular calcium mobilization was used to measure receptor function *in vitro*. In the cells transfected with $CysLT_1$ cDNA, CysLT could increase Ca^{2+} concentration in a rank order potency of $LTD_4 > LTC_4 \geq LTE_4$ [4–7]; or $LTC_4 = LTD_4 > LTE_4$ in cells transfected with $CysLT_2$ cDNA [8–11]. This is consistent with pharmacological studies described previously [3]. Species variations have been described in different studies [6,7,19,20]. For example, the EC_{50} values of LTD_4 for both $mCysLT_1$ isoforms in CHO cells were similar to those for $hCysLT_1$ in COS-7 and HEK-293 cells, while LTC_4 was 1000-fold less potent than LTD_4 at $mCysLT_1$ but only 10-fold less potent at $hCysLT_1$ in one study [6]. In another study, the efficacy of LTD_4 for the long isoform at $mCysLT_1$ was slightly higher than the short isoform, and even higher than the $hCysLT_1$ in HEK-T cells [7]. The variations could come from the intrinsic nature of the receptors, the signaling pathways coupled to the receptors in different cell types, or experimental conditions. The expression level of the receptor could also affect agonist efficacy [21]. Tagging the $mCysLT_1$ with a FLAG epitope not only increased the expression of the receptors but also shifted the EC_{50} value leftward [7]. Potential functional modification of the receptor by the FLAG-tag is possible.

Intracellular signaling pathways of CysLT receptors have not been studied thoroughly. Pertussis toxin did not change LTD_4-induced functional responses in either $CysLT_1$ cRNA injected *Xenopus laevis* oocytes [4] or $CysLT_1$-transfected HEK-293 cells [5], indicating that the receptors in these cells were only coupled to $G_{q/11}$ family members. Early studies [22,23] also showed $G_{q/11}$ coupling in some cell types expressing $CysLT_1$ endogenously. However, in differentiated human U937 cells [24], intestinal 407 cells [25], and human monocytic leukemia THP-1 cells [26], coupling to both the $G_{q/11}$ and the $G_{i/o}$ family has been observed. So, $CysLT_1$ signaling pathways are cell-type dependent, and the availability of G proteins may account, in part, for the differences. Limited information about CysLT responses downstream of trimeric G proteins is known. In THP-1 cells, mitogen-activated protein kinase activity was increased by the PKCα–Raf-1 pathway [26] after $CysLT_1$ stimulation. Receptor activation also increased topoisomerase I activity through PKC in U937 cells, leading to a rapid induction of gene expression [27]. $CysLT_2$ is coupled to the $G_{q/11}$ family in HEK293 transfected cells [8–11]. Whether this receptor has other coupling patterns in other cell types is unknown.

GPCR dimerization, or even oligomerization, is an emerging concept for ligand binding, signal transduction, and intracellular trafficking [28,29]. This phenomenon is especially common in class A GPCRs. Computational method evolutionary trace (ET) predicted functionally important clusters of residues for dimerization on the

external faces of TM5 and 6, and TM2 and 3, and identified ET residues in putative interfaces in the subfamilies [30]. Do CysLT receptors form homodimers or heterodimers with other receptors, and, if so, how does this affect receptor pharmacology and function? Recombinant $hCysLT_1$ expressed in *Escherichia coli* was able to form large amounts of dimerized and oligomerized receptors even in the presence of denaturing gel detergents [31]. How this is relevant to the true cell physiological environment needs to be addressed. The molecular cloning of the receptors has made potential research in this area possible given all the mature methods available, such as co-immunoprecipitation, fluorescence resonance energy transfer (FRET), bioluminescence resonance energy transfer (BRET), and site-directed mutagenesis.

5. Expression and regulation of CysLT receptors

CysLT receptor tissue distribution has classically been linked to binding studies or functional characterization, using tissue preparations, by observing CysLT effects and the reversing actions of $CysLT_1$ specific antagonists or the $CysLT_{1/2}$ dual antagonist BAYu9773. Considering CysLTs as inflammatory mediators and their significant contributions to human asthma, expression patterns were characterized in lung and inflammatory cells [32]. $CysLT_1$ and $CysLT_2$ expression patterns vary considerably in different tissues and different species as reviewed [33,34]. Though the traditional pharmacological methods are very powerful, they also have certain limitations. The most important drawback has been the lack of specific $CysLT_2$ antagonists, which aroused great ambiguity in interpreting functional data, so that the existence of $CysLT_2$ was only conceptual as a subfamily.

Expression of $CysLT_1$ and $CysLT_2$ in human tissues is summarized in Table 1. High expression of $hCysLT_1$ was observed in spleen by northern blot analysis. Peripheral blood leukocytes, lung, pancreas, small intestine, prostate, and a few other tissues also showed defined levels of expression [4,5]. By more sensitive *in situ* hybridization, immunohistochemistry with specific antisera, RT–PCR, or combined methods, detailed expression has been characterized in the lungs and peripheral blood leukocytes [4,16,31]. Both $hCysLT_1$ mRNA and protein are detected in peribronchial and peribronchiolar smooth muscle cells, and interstitial macrophages. Twenty percent of peripheral blood monocytes (PBMC) stained positive for $CysLT_1$ by *in situ* hybridization [31]. These included eosinophils, monocytes/macrophages, B lymphocytes, and $CD34^+$ granulocytic precursor cells. No expression was detected in neutrophils or T lymphocytes by this method, while contradictory data using competitive RT–PCR showed low expression in these two cell types [35]. $CysLT_1$ mRNA and/or protein were also detected in cultured human mast cells [16] and some hematopoietic cell lines consistent with previous functional studies, such as promyelocytic HL-60 [5,36,37], lymphoblastoid U937 [5,20], and monocytic THP-1 cells [35,38]. $mCysLT_1$ has an expression pattern distinct from that of $hCysLT_1$ [6] where it is expressed highly in the lungs and skin and minimally in other tissues. The long transcript is preferentially expressed.

The $hCysLT_2$ expression pattern is substantially different from that of the $hCysLT_1$ [8–10]. It is also highly expressed in spleen and peripheral blood leukocytes, but expression in the heart, adrenal glands, and brain appears unique to this subtype. All four chambers of the heart, the interventricular septum, the apex, and the pericardium have been shown to express $hCysLT_2$ by northern blot [10] and RT–PCR [39]. Expression in Purkinje fiber cells

Table 1
$hCysLT_1$ and $hCysLT_2$ expression patterns

Tissue	$hCysLT_1$		$hCysLT_2$	
	Expression level	References	Expression level	References
Lung				
Bronchial smooth muscle cells	++	[4,5,31]	+	[8]
Interstitial macrophages	++		++	
Peripheral blood leukocytes				
Eosinophils	++		++	
Monocytes/macrophages	+		+	
Neutrophils	±	[4,5,31,35]	+	[8–10,35]
B lymphocytes	+		?	
T lymphocytes	±		+	
CD 34^+ precursor cells	+		?	
Mast cells	+	[16]	?	
Spleen	++	[4,5]	++	[8–10]
Heart and coronary vessels		[4,5,39]	++	[8–10,39]
Brain		[4,5]	++	[8,10]
Adrenal gland	?		++	[8,10]

Key: (++) high expression; (+) low expression; (±) expression questionable; and (?) not examined yet. Grading could be subjective and may not be comparable between different studies.

and coronary smooth muscle was detected by *in situ* hybridization, and $CysLT_2$ mRNA from both human coronary smooth muscle cells (HCASMC) and endothelial cells (HCAEC) was detected by RT–PCR [39]. Expression of $hCysLT_2$ in other vascular tissues, such as in human umbilical vein endothelial cells (HUVEC), was also reported [35]. However, $hCysLT_1$ expression was only detected in HUVEC after cytokine induction [40], but not in any other of the cardiovascular tissues listed above. The identification by molecular methods of $hCysLT_2$ on the vascular walls is not totally surprising, as functional studies have verified its existence; its strong expression in the adrenal glands and the central nervous system was much less anticipated. In the adrenal glands, expression is evident in medullary pheochromocytes and ganglion cells, and also in the zona reticularis adjacent to the corticomedullary junction [8]. In the central nervous system, $CysLT_2$ expression is widespread, including most regions of the brain and spinal cord [8,10]. Expression in peripheral blood leukocytes is also abundant [8]. Eosinophils express higher levels of $CysLT_2$ than $CysLT_1$ as determined by competitive RT–PCR [35]. Expression in monocytes, neutrophils, and T cells was also observed [35]. Compared with $CysLT_1$, $CysLT_2$ is only weakly expressed in lung smooth muscle cells [8], but elevated expression was detected in macrophages in close proximity to smooth muscle cells. Similar expression patterns of $CysLT_2$ were seen in mouse tissues [11]. There are two transcripts of mouse mRNA. The short transcript is the predominant form in every tissue expressing the receptor. The significance of alternative splicing is not clear. Since there is no protein difference between the transcripts, the alternatively spliced exon 3 could serve some regulatory role in transcription or translation.

Modulation studies of CysLT receptor expression are only in the infancy stage. Early binding studies demonstrated that differentiation of U937 cells by dimethyl sulfoxide increased binding sites for LTD_4 [20]. Receptors binding LTD_4 were also induced in HL-60 cells differentiated into eosinophils [36]. Recently, several studies showed up-regulation of CysLT receptors by cytokines [36,40–42]. IL-5 rapidly enhanced $CysLT_1$ mRNA levels and consequently increased receptor numbers in HL-60 cells differentiated towards eosinophils (HL-60/eos), but not in undifferentiated HL-60 cells [36]. It is possible that HL-60/eos cells secreted IL-5 and stimulated CysLT receptor production [37] in an autocrine pathway. IL-13 and IL-4, but not IFN-γ, were also able to induce $CysLT_1$ expression in human monocytes and macrophages [42]. IFN-γ, however, was able to induce both $CysLT_1$ and $CysLT_2$ in human airway smooth muscle (HASM) cells [41]. $CysLT_1$ mRNA in HUVEC could be induced by IL-1β treatment [40]. Therefore, it appears that both T-helper-1 (Th1) and Th2 type cytokines can alter CysLT receptor expression, which could be important in human asthma pathogenesis. Characterization of the promoter regions of CysLT receptor genes will help elucidate signaling pathways and transcription factors involved in receptor modulation.

6. Functions of CysLT receptors

The role of CysLTs in inflammation has been studied intensively, and emphasis has been placed on human asthma. A large market for antileukotriene drugs to treat human asthma symptoms has been successfully established since 1995. CysLT and their receptor function studies are not confined to asthma etiology. In fact, the concept that CysLTs are mainly inflammatory mediators may be modified as evidence of their potential involvement in cardiovascular and neurological modulation is accumulating.

6.1. Asthma

The remarkable contractile activities of LTC_4 and LTD_4 on isolated human bronchi were described in the early 1980s [43], and later, bronchoconstricting effects *in vivo* were also found in humans [44]. Other effects include increased microvasculature permeability by retracting endothelial cells in the post-capillary venules [45,46] leading to pulmonary edema, increased mucus secretion [47] and decreased mucus clearance by impairing cilia activity [48], and eosinophil recruitment. A consensus that CysLTs are one of the final common pathways to promote airway obstruction and inflammation in asthma is evident [49]. $CysLT_1$ is responsible for the preponderance of CysLT airway actions since $CysLT_1$ antagonists can reverse most effects.

Recent asthma research is focused on the chronic inflammation and remodeling of the airway, manifested as airway thickening, increased smooth muscle mass, subepithelial fibrosis and matrix protein deposition, epithelial cell damage and hyperplasia of goblet cells, infiltration of eosinophils in epithelium and submucosa, and cytokine production [50–52]. CysLTs may take part in this remodeling process by their direct effects on smooth muscle cells, eosinophils, epithelial cells, and possibly other cell types. In a mouse ovalbumin-sensitized/challenge asthma model, montelukast, a $CysLT_1$ antagonist, significantly reversed almost all of the morphological aspects of chronic asthma [53]. CysLTs can cause smooth muscle cell proliferation and hypertrophy in conjunction with other known mitogens [54–56]. For instance, LTD_4, did not affect DNA synthesis in HASM cells, but augmented proliferation induced by epidermal growth factor (EGF), which is elevated in asthma [55]. Interestingly, this effect was abolished by pranlukast and pobilukast, but not zafirlukast [56]. All of these chronic pathological changes will ultimately lead to bronchoconstriction and bronchial hyperresponsiveness [57]. The human airway inherent tone is thought to be the balance between contractile forces generated

mainly by CysLTs, and histamine to a lesser extent [58], and relaxing elements (e.g. prostaglandin E_2) [59]. In chronic asthma, the contractile tone is reinforced probably due to smooth muscle hyperplasia and enhanced CysLT production [60,61]. Except for IFN-γ-induced $CysLT_1$ up-regulation in HASM cells [41], there is no direct evidence showing that $CysLT_1$ is up-regulated in the airways of asthmatic patients. If that is the case, it might explain why asthmatics are generally more responsive to CysLTs than nonasthmatics [62], and a coupled increase in CysLTs acting via $CysLT_1$ could lead to more prominent bronchoconstriction.

Eosinophil activity is pivotal to the chronic inflammation associated with asthma. The relationship between CysLT-induced pathophysiology and eosinophil infiltration needs to be addressed in order to further unravel asthma progression. CysLTs are produced mainly by eosinophils but also by mast cells at the rapid onset of allergic asthma. Increased CysLTs in bronchoalveolar lavage and urine [60,61] can be explained as a result of enhanced eosinophil number and activity. On the other hand, eosinophils express $CysLT_1$s on their surfaces that mediate poorly defined CysLT autocrine actions. Inhaled LTD_4 produced dose-dependent airway eosinophil infiltration in guinea pigs that persisted for up to 4 weeks, which could be blocked by pranlukast [63]. Also, CysLT inhalation increased the eosinophil count in the bronchial mucosa [64] and the percentage in sputum in asthmatic patients within 4 hr [65]. A $CysLT_1$ antagonist was found to reduce airway eosinophilia after other antigen challenges [66], indicating the contribution of endogenous CysLTs. In fact, in 5-lipoxygenase-deficient mice lacking the ability to produce CysLTs, there was a 50% reduction in airway eosinophils in an ovalbumin-sensitization/challenge model [67]. Eosinophil recruitment is a combined result of increased maturation and mobilization from bone marrow, functional priming in the circulation, chemotaxis to the sites of action, enhanced interaction with endothelial cells via up-regulating adhesion molecules, interaction with matrix proteins, and enhanced survival [68]. CysLTs are chemoattractants for eosinophils through $CysLT_1$ [69]. CysLTs induced P-selectin on both leukocytes [70] and endothelial cells (possibly through $CysLT_2$ in this cell type) [71], and increased rolling and adhesion of eosinophils to vasculature. CysLTs are important in maintaining eosinophil survival through $CysLT_1$s with a potency as high as granulocyte-macrophage colony stimulating factor (GM-CSF) and fibronectin [72]. Cytokines like GM-CSF and IL-5, and adhesion to the endothelial surface through intercellular adhesion molecule 1 (ICAM-1) [73], or adhesion to matrix proteins through the integrin very late antigen (VLA)-4 [74], stimulate CysLT production, which in turn stimulates the secretion of more cytokines and adhesion molecules from eosinophils. Analogous results were observed in IL-4-primed human mast cells whereby CysLTs stimulated IL-5, tumor necrosis factor (TNF)-α, and macrophage inflammatory protein (MIP)-1β secretion possibly via $CysLT_1$ [75]. Thus, $CysLT_1$ may be up-regulated by various cytokines, adhesion molecules, or even CysLTs, to further potentate CysLT effects.

Th2 lymphocytes are associated with human asthma, basically by secretion of cytokines like IL-5, IL-4, IL-13, as well as eotaxin and GM-CSF, which are important to eosinophil chemotaxis, migration, and survival. T-bet is a Th1-specific T-box transcription factor and is able to modulate a phenotypic Th2 to Th1 cell switch [76]. *T-bet* gene-deficient mice spontaneously displayed chronic asthma features [77]. Whether CysLTs directly regulate T cell function has not been investigated and expression of CysLT receptors in T cells is contradictory [31,35].

6.2. Cardiovascular regulation

CysLTs have negative inotropic effects on the myocardium and decrease coronary blood flow through direct coronary constriction in several species [78,79] with no profound effect on heart rate [80]. $CysLT_1$-specific antagonists could reverse these effects, but individual antagonists may preferentially inhibit some effects [81]. CysLT production is increased in ischemia–reperfusion injury, both in patients with coronary artery disease [82] and in experimental animal models [83]. Inhibition of the 5-lipoxygenase pathway by a FLAP inhibitor provided protection in a rabbit ischemia model [83,84] manifested as reduced mortality rate, myocardial enzyme activity, and arrhythmias. CysLTs also mediate cardiac anaphylaxis induced by platelet activation factor (PAF) [85]. 5-Lipoxygenase-deficient mice [86] showed reduced evidence of mortality and faster normalization of blood pressure from PAF-induced shock. However, LTA_4 hydrolase-deficient mice, which cannot produce LTB_4, were also resistant to PAF-induced shock, leaving the question of whether the combined effects from LTB_4 and CysLTs are a more suitable explanation to this specific model. More detailed cardiovascular parameters need to be measured to differentiate the involvement of LTB_4 and CysLTs. Contrary to the early pharmacological studies implicating $CysLT_1$ in the mediation of cardiac failure, $CysLT_1$ mRNA is not readily detectable in human heart and coronary vessels, while $CysLT_2$ is the major receptor in these regions [39]. Stimulation of HCASMC with LTC_4 elicited calcium mobilization, which was not inhibited by $CysLT_1$ antagonists but by a calcium channel blocker known to be a vascular relaxant. This suggested a role for $CysLT_2$ in mediating coronary constriction. The detection of $CysLT_2$ in various regions of the heart was striking, especially in the conduction system. How CysLTs affect cardiac function through $CysLT_2$ will be answered by receptor deficiency models, if specific antagonists at the receptor are not available in the near future.

CysLTs may modulate vascular tone in specific vascular beds [87]. CysLTs can mediate both vascular contraction

and relaxation. Relaxation usually is endothelium-dependent, while stimulation of receptors on either endothelium or smooth muscle cells can cause contraction. A model of CysLT receptor distribution and function in the human pulmonary vasculature has been formulated by Walch *et al*. [87]. $CysLT_2$ sits on the endothelium of the artery and mediates relaxation. An unknown receptor type on smooth muscle cells mediates contraction. In the pulmonary vein, $CysLT_2$ on the endothelium also mediates relaxation, while $CysLT_1$ on the endothelium and $CysLT_2$ on smooth muscle cells mediate contraction. The model was further modified since a receptor on the endothelium of the artery that is sensitive to neither a $CysLT_1$ antagonist nor a $CysLT_1$/$CysLT_2$ dual antagonist may mediate relaxation by releasing prostacyclin [88], and the same receptor on smooth muscle cells may mediate contraction. Nitric oxide production or cyclooxygenase pathways are thought to be responsible for CysLT-induced endothelium-dependent relaxation in most vascular preparations [89–91].

CysLTs may also be involved in other cardiovascular pathophysiological processes. LTC_4 and LTD_4 induced contraction in human atherosclerotic coronary arteries but not in non-atherosclerotic coronary artery rings [92]. Specific binding of LTC_4 was detected in smooth muscle cells and regions of intimal proliferation and plaque. CysLTs could also modify the effects of other vascular vasoactive agents. Angiotensin II vasoconstrictor effects were enhanced by CysLTs in spontaneously hypertensive rats (SHR) [93] and streptozotocin-induced diabetic rats [94]. This modulation effect is specific since they did not mediate vasoconstriction induced by endothelin-1 in the SHR model [95].

6.3. Neuroendocrine modulation

The expression of $CysLT_2$s in the human brain and the adrenal glands is attracting attention to the largely neglected area of neuroendocrine modulation by CysLTs. *In vitro* studies showed LTC_4 synthesis in most regions of the rat brain stimulated by the ionophore A23187, with the highest levels in the hypothalamus and the median eminence [96,97]. Binding studies showed LTC_4 binding sites throughout the rat brain [98]. LTC_4 could stimulate luteinizing hormone (LH) release from rat anterior pituitary cells [99] and luteinizing hormone-releasing hormone (LHRH) from the median eminence [100] both *in vitro* and *in vivo* [101]. The stimulation was specific for LH and LHRH, and no response was observed in growth hormone (GH), somatostatin (SRIF), and growth hormone-releasing factor (GRF) secretion [99,100]. $CysLT_1$ antagonists did not inhibit the LHRH-releasing effect of LTC_4. The effects of CysLTs on the hypothalamus–pituitary–adrenal gland axis have never been described. Studies in both the cortex and medulla of the adrenal gland may reveal profound effects of CysLTs on endocrinal and neural systems. In addition to possible neuroendocrine regulation, CysLTs may also contribute to neurological inflammation. CysLT production was increased in cerebrospinal fluid in certain disease states (patients with multiple sclerosis [102] and intracerebral hemorrhage [103]), which may exacerbate disease by impairing the blood–brain barrier and causing neuronal edema and death.

7. Prospects

Since their discovery, CysLTs have been regarded mainly as important mediators of inflammation, and research accordingly has been tied to the elucidation of their effects in inflammatory processes. The molecular cloning of the two CysLT receptor subtypes has not only confirmed many previous pharmacological observations but has also broadened the horizon to unexpected physiological effects of CysLTs beyond inflammation and removed a hurdle to elucidate CysLT signal transduction mechanisms. How factors regulate CysLT receptor expression and function in normal physiological states and during disease progression will be important questions to address. What are the downstream effectors of CysLTs and can dysregulation of the signaling pathways contribute to disease pathogenesis? Is there any crosstalk between the two CysLT receptors, especially in eosinophils where they are co-expressed? Do CysLTs and their receptors mediate communication between inflammatory cells and cells intrinsic to the airways and other sites of inflammation? Are there any genetic variants that alter receptor expression or evoke constitutive activation, which might influence responses to current antileukotriene therapies and provide new therapeutic targets? The answers to these questions will yield important insights into inflammatory disease etiology. Lack of specific $CysLT_2$ antagonists has hindered accurate characterization of the receptor and complicated functional interpretation of $CysLT_1$ actions and other potential receptor subtypes. Development of specific antagonists will be simplified with novel receptor molecular modeling studies. Receptor transgenic animal models will provide valuable information *in vivo*. The expression of $CysLT_2$ in the heart, brain, and adrenal glands will open up a largely overlooked area in cardiovascular and neuroendocrine regulation by CysLTs.

Even though both $CysLT_1$ and $CysLT_2$ have been cloned, there is strong evidence for an additional receptor subtype based on pharmacological data. The most expected features of this potential $CysLT_3$ are specificity for LTC_4 and/or resistance to the $CysLT_1$/$CysLT_2$ dual antagonist BAYu9773.

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