

## REVIEW

# Calcium sensors as new therapeutic targets for asthma and chronic obstructive pulmonary disease

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## Introduction

Airway diseases such as asthma and chronic obstructive pulmonary disease (COPD) are characterized by airway hyper-responsiveness, epithelial damage, oxidative stress and airway inflammation. Inflammatory cells like macrophages, neutrophils, eosinophils and lymphocytes are crucial in the pathogenesis of asthma and COPD [1, 2]. The prevalence of asthma and COPD is increasing, especially in Western countries [3]. The symptomatic treatment is nowadays mainly based on reduction of the airway obstruction by inhalation of  $\beta_2$ -receptor agonists and attenuation of the underlying inflammatory reactions in the airways by inhalation of corticosteroids [1, 4]. Although new therapeutic approaches such as anti-leukotrienes, anti-IgE and immunotherapy have been developed, new insights into the pathophysiology of these diseases might lead to other effective therapeutic targets for the treatment of asthma and COPD [5, 6]. Indeed, the regulation of intracellular calcium may play a crucial role in the pathogenesis because an increase in the intracellular calcium concentration ( $[Ca^{2+}]_i$ ) is an essential initiating mechanism of cellular activation in several cells involved in these airway diseases, including inflammatory cells, mast cells, epithelial cells and smooth muscle cells. Processes such as secretion, muscle contraction, cell growth and differentiation, degranulation, release of mediators are all dependent on the  $[Ca^{2+}]_i$ . Tight regulation of  $[Ca^{2+}]_i$  is therefore fundamental to the homeostasis of virtually all cells.

Many different agonists, such as hormones, neurotransmitters and other physiological and pathophysiological stimuli, initiate cellular responses through an increase in cytosolic calcium. The increase is a consequence of calcium release from internal stores and calcium influx through calcium-permeable cation channels in the plasma membrane [7]. Upon receptor activation, inositol 1,4,5-triphosphate ( $IP_3$ ) is formed, which leads to calcium release from the internal stores present in the endoplasmic reticulum (ER). The consequent activation of calcium entry through plasma-membrane channels is caused by an as yet not completely known mechanism [8].

Calcium is a key secondary messenger, whose function is tightly regulated by the calcium-sensing regulatory protein calmodulin (CaM). Because CaM is abundantly present in almost every eukaryotic cell type, modulation of CaM activity

affects many processes in these cells. Ligands that interact with calcium sites on proteins offer the opportunity to modify calcium/CaM-dependent processes. Therefore, specific modulation of CaM function may be a useful target for affecting these processes. In this review we describe the effects of two calcium-like peptides, termed CALP1 and CALP2, which specifically bind to the calcium-binding sites of CaM, the EF-hands. First, the complementary eight-residue peptide CALP1 was synthesized [9]. CALP1 has a hydrophobic profile opposite to the EF-hand motif based on the primordial calcium-binding site of the troponin C superfamily. In 1991, CALP1 was shown to increase CaM-stimulated phosphodiesterase (PDE) activity. Moreover, CALP1 added to the luminal side of the bladder, increased resting and acetylcholine-induced tension in canine urinary bladder smooth muscle. Because the hydrophobic profile of CALP1 is not perfectly inverted relative to the EF-hand motifs of CaM, a second complementary peptide was developed with a more inverted hydrophobic pattern. This 12-mer peptide was called CALP2. Studies by Villain et al. [10] and Manion et al. [11, 12] recently elucidated the characteristics of these peptides. The most striking observation was that CALP1 increased PDE activity, and therefore CALP1 is a CaM agonist. In contrast, CALP2 inhibited calcium/CaM-dependent PDE activity and therefore acts as a CaM antagonist [10]. A second interesting observation was that CALP1 entered rat neocortical neuronal cultures and Jurkat T cells. Moreover, CALP1 blocked the activity of glutamate receptor channels in neurons and a non-selective cation channel in Jurkat T cells. Furthermore, apoptosis mediated by an influx of calcium through these channels was also inhibited by CALP1. These studies therefore show that CALPs most probably have intracellular targets and that modulation of calcium channel functioning is the most predominant mechanism of action.

Here, we review the effects of the two CALPs, CALP1 and CALP2, on several cell types (epithelial cells, mast cells and inflammatory cells) involved in asthma and COPD. Furthermore, we tested whether the peptides can modulate airway responsiveness in guinea-pigs and whether they have effects on the development of asthmatic features in a guinea model of allergic asthma.

## Calcium sensors epithelium-dependent modulate airway hyper-responsiveness

Features of asthma and COPD are epithelial cell damage and airway hyper-responsiveness to a variety of specific and

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non-specific stimuli. The degree of epithelial damage is associated with the degree of airway hyper-responsiveness [13]. The airway epithelium is a physical barrier that protects sensory nerves and smooth muscle from stimulation by inhaled irritants [14]. Epithelial cells also release mediators that can inhibit bronchoconstriction by relaxing the underlying smooth muscle. These 'epithelium-derived relaxing factors', such as prostaglandin E<sub>2</sub> and nitric oxide (NO), protect the airways from excessive bronchoconstriction (reviewed in [15]). Indeed, inhaled NO causes bronchodilation in asthmatic patients [16] and inhibition of NO production by NO synthase inhibitors increases airway responsiveness in both patients with mild asthma [17] and guinea-pigs [18, 19].

Because the constitutively expressed NO synthase (cNOS), present in the airway epithelium [20, 21], is activated by CaM in a calcium-dependent manner [22], we investigated whether CALP1 and CALP2 can modulate airway responsiveness. In anaesthetized guinea-pigs, intratracheal administration of CALP1, but not CALP2, resulted in increased responses to histamine. Using a perfused organ bath set-up, in which selectively the influence of the epithelial layer on contractile

responses can be investigated, CALP1 incubation shifted the histamine concentration–response curve significantly upwards (Fig. 1a). In contrast, CALP2 had no effect on basal responsiveness, but completely inhibited the CALP1-induced airway hyper-responsiveness. To investigate the role of the epithelium, the same experiments were performed with epithelium-denuded tracheas. Neither CALP1 nor CALP2 changed the airway responsiveness when applied to the inside of epithelium-denuded tracheas (Fig. 1b). It was therefore demonstrated that the effects of both peptides are epithelium dependent. Experiments using airway epithelial cells showed that both peptides are rapidly taken up by these cells (Fig. 2a), and therefore the putative sites of action are located within the cell. To explore the mechanism of action of CALP1 and CALP2, [Ca<sup>2+</sup>]<sub>i</sub> and NO production in epithelial cells were measured. CALP1 decreased receptor agonist-induced increases in [Ca<sup>2+</sup>]<sub>i</sub> in epithelial cells. In contrast, CALP2 increased receptor agonist-induced increases in [Ca<sup>2+</sup>]<sub>i</sub>, which was accompanied by an increased NO production by epithelial cells. The CALP2-induced increase in [Ca<sup>2+</sup>]<sub>i</sub> was mainly due to more sustained extracellular calcium influx.

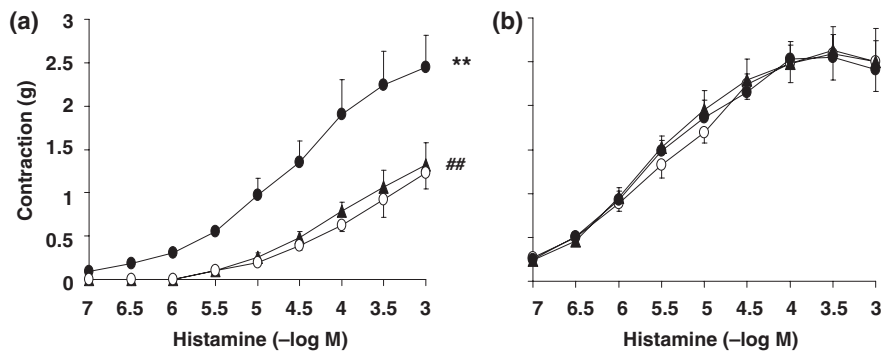


**Fig. 1.** Concentration–response curves of intraluminal administered histamine made on isolated perfused guinea-pig tracheal tubes. (a) The histamine concentration–response curve was significantly ( $**P < 0.01$ ) shifted upwards after intraluminal administration of CALP1 (●). Incubation with CALP2 had no effect on the histamine-induced smooth muscle contraction (○), but CALP2 prevented the CALP1-induced airway hyper-responsiveness to histamine (▲,  $###P < 0.01$  vs. CALP1 alone). (b) The histamine concentration–response curve was shifted upwards in tracheas denuded of epithelium (○). Intraluminal administration of neither CALP1 (●) nor CALP2 (▲) influenced the smooth muscle contraction to histamine. Data are presented as mean  $\pm$  SEM,  $n = 6$ .  $***##P < 0.01$  two-way ANOVA.

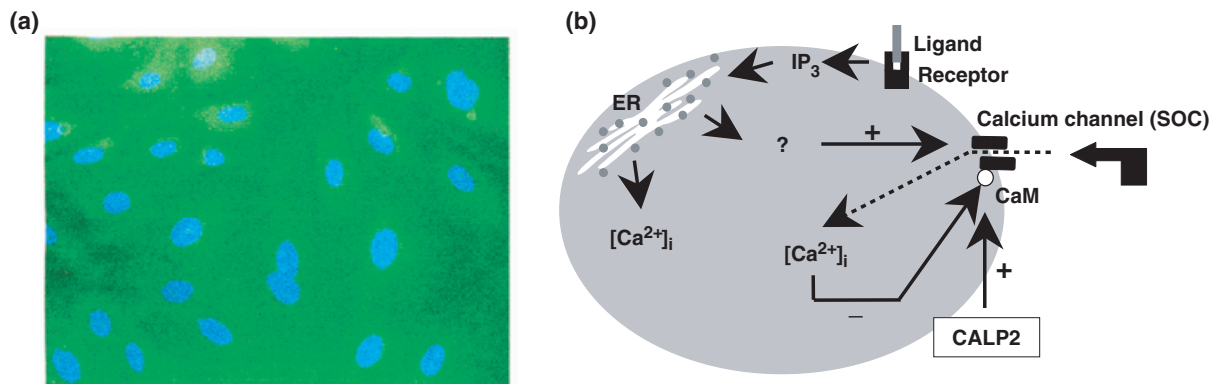


**Fig. 2.** (a) Fluorescent microscopic images of cultured guinea-pig tracheal epithelial cells stained with F-CALP1 (50  $\mu$ M) showed diffuse cytoplasmic staining (green) throughout the cell. Cells were counterstained with Hoechst 33258 (7.5  $\mu$ M, blue) to show the nuclei of cells. (b) Schematic diagram of the hypothesized mechanism of action of CALP2 in non-excitable cells. The CaM antagonist CALP2 binds to CaM, which is bound to calcium channels. Upon binding, CALP2 prevents the binding of calcium to CaM and thereby inhibits the negative feedback, resulting in increased extracellular calcium influx through store-operated calcium channels.

It is tempting to speculate about the mechanism of action of CALP in modulation of airway responsiveness. Interestingly, both peptides specifically act on the epithelium. This could mean that the targets of CALP are only in the epithelium and not in smooth muscle cells. This suggests, however, that targets other than CaM, which are present in both epithelial and smooth muscle cells, play a role in the CALP responses. It is likely that the difference between the two cell types arises from different types of calcium channels. Knowing that CALP2 is a CaM antagonist, incubation of epithelial cells with this peptide was supposed to result in decreased NO production by eNOS in epithelial cells. However, we observed increased NO production by epithelial cells. Moreover, increased  $[Ca^{2+}]_i$  were found upon CALP2 stimulation. We therefore postulated that CALP2 increases  $[Ca^{2+}]_i$  in epithelial cells, which consequently results in the production of NO. CALP1, on the other hand, decreases  $[Ca^{2+}]_i$  and diminishes NO production by epithelial cells (Fig. 3). The observation that both peptides modulate  $[Ca^{2+}]_i$  in epithelial cells, leads us to hypothesize that CALP2 opens calcium channels in epithelial cells, which results in sustained calcium influx and that CALP1 closes these channels and thereby preventing calcium influx (Fig. 2b). Recently, it was found that calcium channels, which show calcium-sensitive inactivation, have EF-hand- and CaM-binding motifs on the cytoplasmic side of the

channel [23]. When intracellular calcium binds these sites, it causes the channel to close or inactivate. We have previously shown that CALP1 blocked a store-operated calcium channel (calcium release-activated calcium current,  $I_{CRAC}$ ) in mast cells [24]. Moreover, Manion et al. [11, 12] showed that CALP1 blocked glutamate receptor channels in neurons and a non-selective cation channel in Jurkat T cells. If CALP influences calcium-permeable channels in epithelial cells, CALP should be able to enter the cell. Using a fluoresceinated derivative of CALP1 and fluorescent microscopy, we observed that the peptide crosses the cell membrane and accumulates in the cytoplasm of epithelial cells (Fig. 2a). In conclusion, we have demonstrated that the CALPs modulate airway responsiveness by affecting  $[Ca^{2+}]_i$  and NO levels in epithelial cells.

### Calcium sensors modulate mast cell adhesion and degranulation

Mast cells are localized in all vascularized tissues and play an important role in type I hyper-sensitivity responses [25]. Antigenic stimulation of mast cells by cross-linking of the high-affinity IgE receptor (FcεRI) leads to release of various mediators, which cause clinical phenomena associated with an allergic response [26]. Adhesion of mast cells to the extracellular matrix-component fibronectin through very late Ag (VLA)-5, an  $\alpha_5\beta_1$  integrin, enhances the release of allergic mediators [27, 28]. On resting cells integrins exist in a low-affinity conformation. By interaction with cation-binding motifs in the extracellular domains of the VLA-5 dimer, magnesium and, more potently, manganese induce a high-affinity state of VLA-5 for fibronectin, an effect that can be antagonized by calcium [29]. The cation-binding motifs are highly homologous to the calcium-binding EF-hand motif of CaM [30].

We investigated the modulation by CALP1 and CALP2 of VLA-5- and FcεRI-mediated adhesion of bone-marrow-derived mast cells (BMMC) to the extracellular matrix component fibronectin, together with the effects of these peptides on allergen-induced mast cell degranulation. First, it was shown that VLA-5-mediated adhesion of BMMC to fibronectin due to FcεRI clustering is CaM dependent, whereas manganese-induced adhesion was CaM independent. Consequently, both CALP1 and CALP2 inhibited adhesion induced by FcεRI clustering. Surprisingly, CALP2 inhibited the CaM-independent manganese-induced adhesion to fibronectin. Using a cell-impermeable derivative of CALP2, we found that the latter effect was due to an extracellular target, probably an EF-hand-like domain in VLA-5, on mast cells. Both peptides had no intrinsic effect on mast cell degranulation, but they attenuated FcεRI-induced mast cell degranulation. Because CALP1 inhibited the specific calcium release-activated calcium current ( $I_{CRAC}$ ) in mast cells, it is the consequent inhibition of extracellular calcium influx through store-operated channels that causes the attenuation of mast cell degranulation.

Because FcεRI-induced adhesion as well as degranulation are both dependent on extracellular calcium and activation of CaM, it is plausible to assume that both cell functions are induced by the same mechanism and therefore affected similarly by CALP1 and CALP2. Inhibition by CALP2 seemed predictable as this peptide is an antagonist for CaM

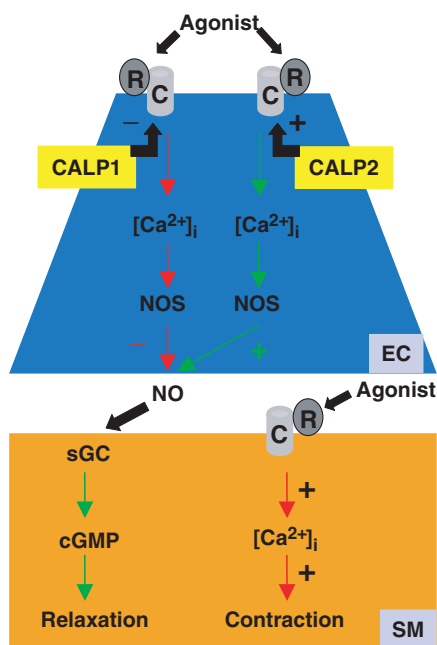


**Fig. 3.** Schematic diagram of the hypothesized action of CALP1 and CALP2 on airway responsiveness in the guinea-pig trachea. In normal situations, there is a balance between calcium influx in the smooth muscle (SM) leading to a contraction on one hand and calcium influx in the epithelial cell (EC) that results in production of nitric oxide (NO), leading to relaxation of the smooth muscle on the other (biochemical antagonism). In cases of NO deficiency or epithelial damage this route of antagonism is disrupted, which leads to an enhanced smooth muscle contraction. CALP2 selectively increases the  $[Ca^{2+}]_i$  in epithelial cells, resulting in the release of NO from the epithelium, which relaxes the smooth muscle and thereby decreases airway hyper-responsiveness. CALP1 on the other hand decreases  $[Ca^{2+}]_i$  in epithelial cells and therefore NO production is diminished. This leads to airway hyper-responsiveness. R, receptor; C, calcium channel;  $[Ca^{2+}]_i$ , intracellular calcium concentration; NOS, nitric oxide synthase; sGC, soluble guanylate cyclase; cGMP, cyclic guanosine monophosphate.

activation [10] and should therefore have the same effect as the CaM inhibitor W7. Inhibition of adhesion and degranulation by CALP1 cannot be explained by inhibition of CaM, since this peptide mimics calcium by activating CaM [9, 10]. Importantly, CALP1 not only mimics calcium in its binding and activating characteristics toward CaM, but is also able to block non-selective cation channels of Jurkat cells [11]. This channel was shown to play a role in calcium mobilization in mast cells [31] and could serve as target for CALP1 in our cells. Additionally, we showed that CALP1 reduces calcium release-activated calcium current ( $I_{CRAC}$ ), which plays an important role in Fc $\epsilon$ RI-induced mast cell degranulation [32]. The closure of cation channels also explains the lack of the intrinsic ability of CALP1 to induce degranulation, which is characteristic of calcium ionophores [33].

In conclusion, we show that these peptides can be applied to block cell adhesion by interfering with CaM and other targets, presumably the EF-hand-like domain of VLA-5. Their ability to block adhesion and thereby modulate the activity of mast cells indicates that these peptides could serve as possible lead compounds for the development of future therapies against allergy and other pathologies in which proteins with EF-hands are involved.

### Calcium sensors modulate superoxide production by alveolar macrophages

Airway inflammation is a characteristic feature in airway diseases such as asthma and COPD. Inflammatory cells like macrophages, neutrophils, eosinophils and lymphocytes are crucial in the pathogenesis of asthma and COPD [1, 2]. Macrophages produce oxygen radicals, such as superoxide anion ( $O_2^-$ ), which contributes to the pathogenesis of allergic reactions, epithelial cell damage as seen in asthma as well as an oxidant/anti-oxidant imbalance in COPD [34]. The most predominant radical  $O_2^-$  is released during the respiratory burst of granulocytes and macrophages by NADPH-oxidase activity, in response to several stimuli [35]. Superoxide rapidly reacts with NO, yielding the highly reactive oxygen radical peroxynitrite (ONOO $^-$ ), which causes tissue injury and induces airway hyper-responsiveness [36].

Recently, we used CALP1 and CALP2 to investigate the importance of CaM in radical production. For that purpose, radical production by bronchoalveolar lavage (BAL) cells from guinea-pigs was measured using lucigenin-enhanced chemiluminescence. CALP2, but not CALP1, is a potent and selective activator of guinea-pig alveolar macrophages. CALP2 only slightly activated neutrophils, but did not affect eosinophils. The CALP2-induced radical production was completely blocked by superoxide dismutase and the NADPH-oxidase inhibitor diphenylene iodonium chloride (DPI), showing that superoxide anion, released upon activation of NADPH-oxidase is the main radical produced by CALP2 stimulation. The increased superoxide production was accompanied by an increase of  $[Ca^{2+}]_i$  in alveolar macrophages and was completely blocked by the CaM antagonist W7 and partly by the IP $_3$  receptor antagonist 2-aminoethoxydiphenyl borate (2-APB) and the store-operated calcium channels blocker lanthanum, but not by voltage-dependent calcium channel blockers. Taken

together, CALP2 stimulates superoxide production by alveolar macrophages due to increased  $[Ca^{2+}]_i$ , both from intracellular IP $_3$ -sensitive stores and extracellular influx through lanthanum-sensitive calcium channels.

Previously, we showed that CALP2 also increases  $[Ca^{2+}]_i$  and NO production in airway epithelial cells [37]. It was hypothesized that CALP2, as a CaM antagonist, opens calcium channels in airway epithelial cells, leading to calcium influx in these cells [23, 38]. Therefore, we now postulate that the production of radicals by CALP2 in macrophages is predominantly due to an increase of  $[Ca^{2+}]_i$ . Although less than in the presence of extracellular calcium, macrophages still produce radicals upon stimulation with CALP2 in calcium-free medium. This production was accompanied by a much smaller increase in  $[Ca^{2+}]_i$  than seen in the presence of extracellular calcium, indicating that extracellular calcium indeed plays a role in the effect of CALP2 on radical production in alveolar macrophages. However, CALP2 increased  $[Ca^{2+}]_i$  in the absence of extracellular calcium, suggesting that CALP2 also stimulates calcium release from intracellular stores. It is likely that CALP2 first releases calcium from intracellular stores leading to the initial high radical production and secondly, CALP2 opens store-operated calcium channels, which results in a further increase in  $[Ca^{2+}]_i$ , with the consequent more sustained production of radicals (Fig. 2b). This hypothesis is confirmed by the fact that lanthanum, a selective inhibitor of store-operated calcium channels ( $I_{CRAC}$ ) [39], inhibited the action of CALP2. Furthermore,  $I_{CRAC}$  channels are present in macrophages and neutrophils but not in eosinophils [7]. Because CALP2 did not activate eosinophils, this further strengthens the hypothesis that CALP2, at least partly, acts via (store-operated) calcium channels.

Although CALP2 itself is an inhibitor of CaM, the CaM inhibitor W7 completely blocked the effects of CALP2. First this can be explained by a direct effect of W7 on NADPH oxidase. In this case, W7 blocks the CALP2-induced radical production by inhibiting the effect of CALP2-released calcium on NADPH oxidase. Secondly, W7 can inhibit the CALP2-induced increase in  $[Ca^{2+}]_i$  through blockage of the effects of CALP2 on store-operated calcium channels. We earlier showed that CALP2 did not bind to CaM in the hydrophobic core, which is typical for W7 [40], but rather interferes with the carboxyl-terminal calcium-binding site of CaM [10]. Moreover, it was recently shown that, depending on the protein bound to CaM, CaM undergoes marked different conformational changes, leading to changed calcium binding [41]. It is therefore assumed that W7 inhibits the action of CALP2 through a different inhibitory mechanism on CaM, leading to altered conformational changes.

In conclusion, we found that CALP2 is (1) a strong activator of alveolar macrophages, (2) a mild activator of alveolar neutrophils and (3) is unable to activate alveolar eosinophils. Because CALP2 is a selective CaM antagonist, the present study provides evidence that CaM regulates the production of reactive oxygen species by alveolar macrophages. Further, the calcium-binding site of CaM is likely to have stimulatory effects, whereas the hydrophobic core of CaM has inhibitory effects on radical production. Elucidation of the exact role of CaM in radical production may contribute to the development of new therapeutic strategies for the treatment of inflammatory diseases.

## Calcium sensors modulate asthmatic symptoms in a model for allergic asthma

As mentioned above, CALP1 and CALP2 (1) epithelium-dependently modulate airway responsiveness [37], (2) attenuate mast cell degranulation *in vitro* [24] and (3) modulate superoxide production by alveolar macrophages [42]. Taken together, the CALPs, by modulating CaM-dependent responses, have several effects on different cell types involved in airway responses. Therefore, we used these peptides to define the role of the calcium-sensing regulatory protein CaM in an animal model for allergic asthma. Sensitized guinea-pigs were intratracheally pre-treated with CALP1 or CALP2, and 30 min later the animals were challenged with aerosolized ovalbumin (OVA). We measured acute bronchoconstriction as well as characteristic features of asthma 6 and 24 h after challenge. Neither CALP1 nor CALP2 prevented the anaphylactic response elicited by OVA challenge. However, CALP1 pre-treatment attenuated the influx of inflammatory cells in the lungs 6 h after challenge. Furthermore, radical production by these cells was diminished both 6 and 24 h after challenge. Moreover, CALP1 completely inhibited airway hyper-responsiveness *in vitro* 24 h after challenge. We conclude that CALP1, as a selective CaM agonist, inhibits the development of asthmatic features probably via the attenuation of mast cell degranulation and radical production [43].

The mast cell is thought to be the primary cell in the orchestration of inflammation in the airways [44]. Inhibition of mast cell mediator release is a key target for new therapeutic approaches for asthma. CALP1 and CALP2 both inhibited mast cell degranulation *in vitro* [24]; however, we did not observe a direct effect of the CALPs on the anaphylactic response due to OVA challenge. Therefore, CALP1 and CALP2 seem to have no acute effect on mast cell degranulation *in vivo*. It cannot be excluded that a more chronic inhibiting effect on mast cell degranulation might occur. Indeed, the influx of inflammatory cells 6 h after OVA challenge was attenuated in these animals. Moreover, BAL cells from CALP1-treated animals showed attenuated radical production upon PMA stimulation 6 h after OVA challenge. After CALP2 pre-treatment, we observed no differences in the number of inflammatory cells in the lungs or the activity of these cells compared with untreated guinea-pigs. It is likely that CALP1 inhibits the release of chemotaxins or chemokines by mast cells. These mediators do not have a role in the direct bronchoconstriction, but are important for the recruitment and activation of inflammatory cells in the lungs [44].

CALP1 pre-treatment completely prevented the development of airway hyper-responsiveness 24 h after OVA challenge, but had no effect on the number of inflammatory cells in the lungs. Moreover, CALP1 treatment also decreased radical production by inflammatory cells at this time-point. Although we cannot completely exclude effects of CALP1 on other cell types, these effects might be due to an inhibition by CALP1 of the release of chemotaxins and chemokines by mast cells. We show that CALP1 attenuates the development of airway hyper-responsiveness but has no effect on airway inflammation. Because inflammatory cells produce several cytokines, chemokines and reactive oxygen species, these findings show that not the number of inflammatory cells, but

the activity of these cells [45, 46], determines the development of airway hyper-responsiveness.

Taken together, this study demonstrates that both mast cells and inflammatory cells are crucial in the development of airway hyper-responsiveness. Furthermore, the activity of inflammatory cells seems to play a much more important role in the development of airway hyper-responsiveness than the absolute number of inflammatory cells in the lungs.

## Conclusions

In this review, we describe the effects of CALP1 and CALP2 on several cell types involved in asthma and COPD. CALP1 blocks mast cells mediator release, inhibits the release of epithelium-derived relaxing factors and presumably attenuates radical production by alveolar macrophages *in vivo*. CALP2, on the other hand, blocks mast cell mediator release, increases the release of NO by airway epithelial cells and markedly increases radical production by alveolar macrophages *in vitro*, and most likely also *in vivo*. Taken together, these peptides have both beneficial and detrimental effects on cells involved in the pathogenesis of airway diseases (Fig. 4).

CALP predominantly acts via modulation of (store-operated) calcium channel activity in airway epithelium, mast cells and inflammatory cells. Because increasing evidence shows that altered calcium channel functioning plays an important role in several diseases [41, 47–49], including asthma [50], these channels could also contribute to the pathophysiology of asthma. Indeed, since  $I_{CRAC}$  is important for both mast cell and macrophage activation, selective blockers of this current might be of use in asthma [51]. Moreover, calcium flux through store-operated channels is a common response in leucocytes stimulated by several inflammatory mediators. Inhibitors of CRAC channels therefore have the potential to be useful as immunomodulatory and

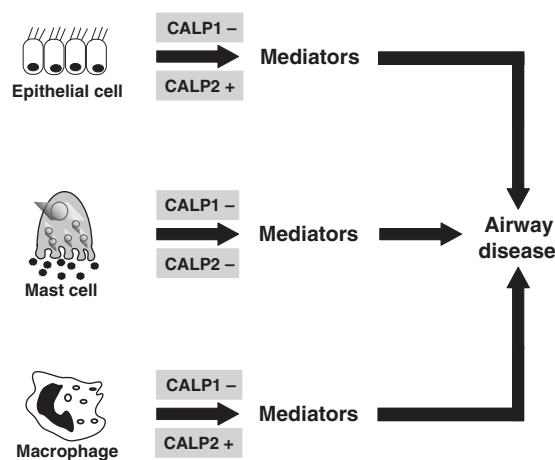


**Fig. 4.** Schematic representation of the proposed effects of CALP1 and CALP2 on different cell types involved in the pathogenesis of asthma. CALP1 blocks mast cell mediator release *in vitro*, inhibits the release of inflammatory mediators by macrophages *in vivo* and direct administration induces airway hyper-responsiveness through the inhibition of nitric oxide (NO) release by airway epithelial cells. CALP2 blocks mast cell mediator release *in vitro*, increases the production of reactive oxygen species by macrophages *in vitro* and attenuates airway hyper-responsiveness through the release of NO by epithelial cells.

anti-inflammatory agents. They could show selectivity because the number of CRAC channels is upregulated in activated or primed lymphocytes [52]; if so, they might be of use in the treatment of asthma. Because  $I_{\text{CRAC}}$  is important for mast cell activation, there could also be therapeutic potential for an  $I_{\text{CRAC}}$  blocker in allergic conditions. Inflammatory diseases in which macrophages or neutrophils are important cells (COPD) are also potential targets, since  $I_{\text{CRAC}}$  is involved in macrophage [53] and neutrophil activation [54].

We conclude that, due to both beneficial and detrimental effects, neither CALP1 nor CALP2 seem to be candidates for therapeutic interventions in asthma and COPD. However, although more research has to be performed to elucidate the exact mechanism of action of these peptides, some indirect evidence has been obtained that store-operated calcium channels are crucial in this mechanism. CALPs can serve as useful tool to further explore the role of these calcium channels in airway diseases.

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