Novel Strategies in the Treatment of COPD
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General Discussion
According to the Global Initiative for Chronic Obstructive Lung Disease (GOLD), chronic obstructive lung disease (COPD) is characterized by “persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases” (GOLD 2015). The formation of oxidative stress is believed to play a central role in the pathophysiology of COPD (Barnes 2004; Kirkham and Barnes 2013).

Signaling by cAMP plays an important role in regulation airway smooth muscle (ASM) contraction and proliferation that are also involved in the pathophysiology of COPD (Billington et al. 2013). A-kinase anchoring proteins (AKAPs) enable compartmentalized cAMP signaling by forming a complex with receptors (including β₂-adrenoreceptors), cAMP effectors, phosphodiesterases (PDEs) and other downstream targets (Logue and Scott 2010; Dekkers et al. 2013). In this thesis, we studied the role of AKAPs in regulating ASM plasticity by analyzing the effects of the AKAP-PKA complex disrupting peptide st-Ht31 on markers of the proliferative and contractile ASM phenotypes.

Given the important role of oxidative stress in the pathophysiology of COPD, compounds with anti-oxidant properties could be candidate drugs for the treatment of COPD. A series of Sul compounds (Sul-90, Sul-121, Sul-127 and Sul-136) were developed as such candidates for the pharmacotherapy of COPD. These compounds were previously shown to exert promising cell protection against damage induced by hypothermia and rewarming due to their anti-oxidative properties (Van der Graaf et al. 2014). In this thesis, we screened these Sul compounds for anti-inflammatory and bronchodilatory properties to test which of these compounds have therapeutic potential for the treatment for COPD. The best candidate of these Sul compounds, Sul-121, was used for further study in a lipopolysaccharide (LPS)-induced guinea pig model of neutrophilia and airway hyperresponsiveness (AHR) that mimics symptoms of COPD. In addition, the molecular mechanism of action of Sul-121 was studied in detail.

**AKAPs: Impact on ASM Proliferation and Contractility**

Although many cellular elements play a role in the pathophysiology of COPD, many of the current COPD medications target either the G protein-coupled receptors (GPCRs: e.g. β₂-agonists and anticholinergics) or their downstream signals (e.g. PDE4 inhibitors) (Caramori and Adcock 2003; Salon et al. 2011; GOLD 2015). As one of the best studied signal transduction pathways involving GPCRs, the cAMP signaling pathway mediates a range of diverse cellular events in relation to airway function (Grandoch et al. 2010). Activation of β₂-adrenoceptors stimulates adenylyl cyclase (AC), thereby enhancing the biosynthesis of the second messenger cAMP from ATP. Subsequently, cAMP regulates diverse cellular functions by activating its downstream effector proteins, such as protein kinase A (PKA) and exchange
protein directly activated by cAMP (Epac) (Grandoch et al. 2010; Schmidt et al. 2013). On the other hand, cAMP signaling is terminated by the action of PDEs, which degrade cAMP into 5’AMP (Taskén and Aandahl 2004). Several physiological responses are regulated due to the phosphorylation by cAMP-dependent PKA, including the relaxation of ASM (Billington et al. 2013) and antagonism at the level of pro-inflammatory transcription factors (such as nuclear factor (NF)-κB) (Oldenburger et al. 2012).

It is has become evident that spatio-temporal regulation of cAMP signaling within individual cells is important for proper physiological responses to cAMP elevation. PDEs represent one mechanism by which this spatio-temporal regulation is achieved. In addition, several studies indicate that the communication between receptors, cAMP effectors, PDEs and other downstream targets are regulated by AKAPs, thereby representing another mechanisms of spatio-temporal regulation (Wong and Scott 2004; Dekkers et al. 2013). AKAPs are a group of scaffolding proteins with the ability to associate with PKA via a short α-helical structure. AKAPs act as targeting devices that assemble a large variety of structural and signaling molecules and thereby help those signaling elements to target to different microdomains in cells (Wong and Scott 2004). As indicated by their name, all AKAPs bind to the regulatory subunits of PKA through a conserved short α helical structure, allowing guidance to different sub-cellular domains (Pawson and Scott 1997). Based on this feature, a cell permeable inhibitor peptide, stearated (st)-Ht31, was developed to mimic this short α helical structure, thereby blocking the interaction between all members of the AKAP family and PKA (Vijayaraghavan et al. 1997).

ASM cells are considered to have phenotypic plasticity, allowing it to possess proliferative and/or contractile phenotypes depending on the presence of exogenous stimuli (Halayko et al. 2008). This phenotypic plasticity of ASM plays an important role in the pathophysiology of obstructive pulmonary diseases, such as COPD and asthma, by enabling increased ASM contractility and mass (Halayko et al. 2008). Both processes contribute to airway narrowing and airflow limitation (Amrani and Panettieri 2003; Chung 2005). The hypercontractile phenotype is characterized by an increased expression of contractile proteins, such as α-smooth muscle actin (SMA) and calponin (Halayko et al. 2008). We now demonstrate that long-term treatment with the AKAP-PKA interaction disruptor st-Ht31 increased the contractility of ASM strips. Moreover, st-Ht31 was able to increase expression of typical contractile proteins, such as α-SMA, both in intact ASM strips and cultured ASM cells (Chapter 3). Notably, it seems that the effect of st-Ht31 on contractile protein expression occurs on a post-translation level, as st-Ht31 does not increase mRNA transcription of α-SMA and calponin. In support, blocking RNA synthesis using actinomycin D or inhibiting protein synthesis using cycloheximide did not prevent the increased α-SMA and calponin protein expression induced by st-Ht31 (Chapter 3). Indeed, AKAPs were recently reviewed as factors affecting protein stability by involving the ubiquitin-proteasome system (Rinaldi
et al. 2015). In this system, proteins are first marked with ubiquitin molecules, then later are degraded through the proteasome (Ciechanover 2005). In Chapter 3, we report that calponin stability seems to be affected by proteasomal degradation, as basal calponin expression was induced in the presence of proteasomal degradation inhibitor MG-132. Moreover, MG-132 did not further increase the st-Ht31-induced expression of calponin, suggesting that st-Ht31, like MG-132, can inhibit proteasomal degradation (Chapter 3). In addition, by inhibiting proteasomal activity, MG-132 has been shown to increase the accumulation of ubiquitin-tagged proteins (Ding et al. 2007). Similarly, using Co-IP, we have shown that treatment with st-Ht31 increases ubiquitin-tagged α-SMA protein (Chapter 3). However, treatment of ASM cells with MG-132 alone did not significantly increase basal α-SMA protein, suggesting that the stability of α-SMA protein is not mainly dependent on proteasomal degradation. Thus, st-Ht31-induced increase in α-SMA protein expression may not be primarily dependent on the inhibitory effects of st-Ht31 on proteasomal activity, although it did induce the accumulation of ubiquitinated α-SMA protein.

Exposing ASM cells to growth factors leads to the phosphorylation (and activation) of p70S6 kinase (p70S6K) (Scott et al. 1996; Karpova et al. 1997; Grewe et al. 1999; Roscioni et al. 2011), which upregulates the expression of cyclins such as cyclin D1 (Takuwa et al. 1999; Ravenhall et al. 2000; Chambard et al. 2007). Subsequently, cyclin D1 combines with pre-existing cyclin-dependent kinases (CDKs) to phosphorylate target proteins, such as retinoblastoma protein (Rb), thereby allowing progression of cell proliferation (Lundberg and Weinberg 1998). As mentioned above for ASM phenotypic plasticity, ASM cells exhibit (depending on the presence of exogenous stimuli) distinct proliferative and/or contractile phenotypes (Halayko et al. 2008). Indeed, we showed that st-Ht31, beside increased contractile markers, could increase several typical proliferative markers including DNA synthesis, p70s6K phosphorylation, cyclin D1 expression and Rb phosphorylation (Chapter 3). While exhibiting increased contractility, those st-Ht31 treated ASM strips also showed increased protein expression of proliferating cell nuclear antigen (Chapter 3), a protein that is expressed during the S phase of the cell cycle (Leonardi et al. 1992). Interestingly, despite the increased S phase activity, we reported that st-Ht31 treatment did not induce an increase in cell number (Chapter 3), suggesting that st-Ht31 induces simultaneously a cell cycle arrest after the S phase. Notably, we found that the treatment of st-Ht31 is correlated with a significant downregulation of AKAP8 (Chapter 3). AKAP8, identified to reside in the nucleus, regulates DNA replication and the expression of several proteins known to be involved in the cell cycle regulation (Coghlan et al. 1994; Eide et al. 1998; Han et al. 2015). Although we did not study how st-Ht31 leads to the downregulation of AKAP8, we hypothesize that this process may be responsible for the increased S phase activity and cell cycle arrest in ASM cells. On one hand, AKAP8 was found overlaid with the CDK4 binding site on cyclin D, suggesting that AKAP8 may compete cyclin D1 binding to CDK4 (Arsenijevic et al. 2006).
Therefore, downregulation of AKAP8 could facilitate the interaction of cyclin D1 and CDK4, thereby increasing Rb phosphorylation and S phase activities. On the other hand, AKAP8 has been found to regulate M phase events, such as chromatin condensation, by interacting with the DNA and other proteins, such as a condensin complex component, Eg7 (Collas et al. 1999; Steen et al. 2000), and histone deacetylase 3 (HDAC3) (Li et al. 2006). Therefore, downregulation of AKAP8 could lead to the dysregulation of the M phase, resulting in a cell cycle arrest in the S phase.

In conclusion, st-Ht31 leads to a simultaneous increase in hypercontractile and hyperproliferative markers in both ASM cells and intact ASM strips. The overall functional effect is increased ASM contractility, without alterations in ASM cell number. We believe that our results could have major implications in obstructive respiratory disease, such as COPD, since such patients very often experience both ASM hyperproliferation and hypercontractility at the same time (Lambert et al. 1993; Chung 2005; Bentley and Hershenson 2008; Chung 2008).

**Sul Compounds as Potential Novel Treatment for COPD**

Two of the most important features of chronic obstructive pulmonary disease (COPD) are chronic inflammation and airflow limitation. Next to smoking cessation, the most recommended pharmacological treatments are bronchodilators, including β₂-adrenoceptor agonists (β₂-agonists), and anti-inflammatory medications, such as glucocorticoids (GOLD 2015). Since these drugs are not always effective and particularly resistance to glucocorticosteroids in COPD patients is a major clinical problem, we screened the novel anti-oxidative Sul compounds for their anti-inflammatory as well as bronchodilatory actions in vitro, to investigate their potential usefulness for the treatment of COPD. In Chapters 5 it was found that out of the four Sul compounds tested, Sul-90 and Sul-121 effectively reduced CSE-induced IL-8 production by human ASM cells by approximately 90%, indicating an potent anti-inflammatory effect of these drugs. As a positive control (Wang et al. 2009; Poppinga et al. 2015; Wang et al. 2015), a similar effect was found by the β₂-agonist fenoterol, whereas the other two Sul compounds were either inactive (Sul-127) or even enhanced the CSE-induced IL-8 release (Sul-136). In Chapter 5, we also reported that Sul-90 decreased cell viability at higher concentrations, whereas Sul-121 as well as the two other Sul compounds did not have an effect on cell viability at any of the tested concentrations.

To determine potential ASM relaxing properties of the Sul compounds, we tested the effect of these compounds on ASM strip preparations precontracted with methacholine, and compared these effects with the classical β₂-agonist isoprenaline (Chapter 5). We demonstrated that out of the four tested Sul compounds, Sul-90 and Sul-121 induced ASM relaxation. The maximum relaxation at the highest concentration tested induced by Sul-121 was about 70% of that induced by isoprenaline, whereas Sul-90 induced about 30% relaxation.
To study whether the relaxing properties of the Sul compounds are mediated through activation of $\beta_2$-adrenoceptors, we applied the subtype-nonselective $\beta$-adrenoceptor antagonist, propranolol (Chapter 5). As expected, propranolol significantly shifted the isoprenaline-induced relaxation to the right. The relaxation induced by Sul-90 or Sul-121, however, was unaffected, demonstrating that Sul-90 and Sul-121 induce relaxation of ASM independent of the $\beta_2$-adrenoceptors.

Taken together, these findings indicate that Sul-90 and Sul-121 are unique new anti-inflammatory compounds with bronchorelaxing properties that could be useful for novel treatment of COPD. This is particularly important as currently there is no effective anti-inflammatory therapy for this disease (Barnes 2013). In addition, the potential bronchodilator effect of these drugs may add to their anti-inflammatory action. Of the two compounds, Sul-121 seems to be the most promising candidate. Although it has similar anti-inflammatory effects as Sul-90, it has better bronchodilatory properties and – in contrast to Sul-90 – does not affect cell viability. Thus, our data in Chapter 5 indicate that Sul-121, out of the four Sul compounds being analyzed, is the most promising candidate for a novel treatment of COPD.

**Sul-121: Potential Mode of Action**

Having identified Sul-121 [6-hydroxy-2,5,7,8-tetramethylchroman-2-yl(piperazin-1-yl) methanone hydrochloric acid] as a potential candidate for the treatment of COPD, we studied Sul-121 in a guinea pig model of LPS-induced AHR and neutrophilia *in vivo*. Data in Chapter 6 show that intranasal LPS instillation induced AHR against histamine and increased the number of neutrophils in the BALF as well as in cartilaginous and non-cartilaginous airways, strongly supporting the validity of our model. We showed that pretreatment with inhaled Sul-121 was able to dose-dependently prevent the LPS-induced AHR towards histamine *in vivo*, which was associated with inhibition of the LPS-induced neutrophilia. Since we showed in Chapter 5 that Sul-121 reduced methacholine-induced ASM contraction as well as IL-8 release, it is tempting to explain the protective effect of Sul-121 on AHR by both a bronchodilatory and anti-inflammatory effect. However, the drug appeared to have no effect on basal airway reactivity in this model. Moreover, the actual concentration of Sul-121 in the airways of the treated animals *in vivo* was most likely lower than that causing the relaxation of ASM *in vitro*. Therefore, we assume that the protective effect of Sul-121 on LPS-induced AHR was primarily caused by its anti-inflammatory effect on neutrophilia. Indeed, it has been established that neutrophil infiltration is associated with AHR in COPD patients (van den Berge et al. 2012).

As a potent neutrophil chemoattractant and activator (Hoenderdos and Condliffe 2013), IL-8 levels are positively correlated with neutrophil numbers in COPD patients and in animal models (Keatings et al. 1996; Mio et al. 1997; Yamamoto et al. 1997; Tanino et al. 2002; Zhang et al. 2011; Smit et al. 2013). Under cigarette smoke exposure, increased IL-8 release has
been described for inflammatory cells (Yang et al. 2006; Mortaz et al. 2008) and pulmonary resident cells (Mio et al. 1997; Numanami et al. 2003), including ASM cells (Oltmanns et al. 2005; Oenema et al. 2010). In accordance, the *in vitro* studies in Chapters 5 and 6 showed that Sul-121 dose-dependently reduced CSE-induced IL-8 release from ASM cells, which could explain the inhibitory effect of Sul-121 on LPS-induced neutrophilia (Chapter 6).

We studied NF-κB as a potential target for Sul-121, since NF-κB is involved in the transcription of a variety of cellular genes that regulate the inflammatory response upon producing cytokines, chemokines and cell adhesion molecules (Tak and Firestein 2001; Lawrence 2009). Activation of NF-κB is featured with translocation of its subunit p65 into the nucleus thereby triggering downstream gene transcription (Chandel et al. 2000). Several studies demonstrated that CSE-induced transcription of IL-8 is regulated by NF-κB (Yang et al. 2006; Oenema et al. 2010; Oldenburger et al. 2012). Interestingly, Sul-121 effectively prevented CSE-induced NF-κB activation in ASM cells, measured by the nuclear translocation of the NF-κB subunit p65 (Chapter 6). Our current findings therefore indicate that Sul-121 prevents airway neutrophilic inflammation most likely by decreasing IL-8 release upon inhibition of NF-κB activation in pulmonary resident cells such as ASM cells (Figure 1).

Hydrogen sulfide (H$_2$S) is a gaseous mediator that forms a promising target for new drug development due to the growing evidence of its important physiological role. Serum H$_2$S levels positively correlate with the lung function as indicated by an improved peak expiratory flow rate and peak inspiratory flow rate, and negatively correlate with the levels of neutrophils in bronchoalveolar fluid (Chen et al. 2009). H$_2$S has been shown to be protective against inflammation in airway diseases such as asthma and COPD (Esechie et al. 2008; Li et al. 2008; Chen et al. 2011; Han et al. 2011; Faller et al. 2012; Zhang et al. 2013). Serum H$_2$S levels can be lowered by cigarette smoke in both acute exacerbations of COPD and in healthy control subjects (Chen et al. 2008). In accordance, our data in Chapter 6 indicate that LPS induced a trend towards a decrease in serum H$_2$S levels. Data in Chapter 6 also showed a negative correlation between blood serum H$_2$S level and lung neutrophils. Others reported that blood H$_2$S can be oxidized by the oxidative stress generated by LPS activated neutrophils (Mitsuhashi et al. 2005). Therefore, we speculate Sul-121 might affect serum H$_2$S by lowering its peroxidative conversion via decreasing oxidative stress, presumably caused by neutrophils. We did not find significant effects of Sul-121 on blood serum H$_2$S levels (Chapter 6). Thereby, we assume that the small changes of H$_2$S after treatment with Sul-121 are most likely only an indirect consequence (via reduction of neutrophilia), instead of the major working mechanism, of Sul-121.
Oxidative stress has been proposed to play a central role during the development of the COPD. The data in Chapter 6 suggested that Sul-121 prevented LPS-induced airway inflammation and AHR by targeting oxidative stress. Indeed, Sul-121 reduced cellular reactive oxygen species (ROS) production provoked by phorbol 12-myristate 13-acetate (PMA; Chapter 6). More importantly, in accordance with the decreased airway inflammation and AHR, Sul-121 decreased LPS-induced levels of malondialdehyde (MDA; Chapter 6), a product of peroxidative breakdown of polyunsaturated fatty acids (Ayala et al. 2014). The antioxidant role of Sul-121 was also supported by in vitro studies. Nuclear factor erythroid 2-related factor 2 (Nrf2) is a nuclear factor that controls cellular anti-oxidative responses (Itoh et al. 1997). During the resting state, the activity of Nrf2 is suppressed by binding to Keap1 thereby preventing its nuclear translocation. Under oxidative stress, Keap1 dissociates from Nrf2 thereby allowing its nuclear translocation (Kobayashi et al. 2004). Subsequently, Nrf2-induced anti-oxidative elements start to alleviate oxidative stress responses (Figure 1) (Itoh et al. 1999). The in vitro data in Chapter 6 showed that CSE induced oxidative...
stress, measured by increased Nrf2 nucleus translocation in ASM cells. Importantly, Sul-121 significantly decreased CSE-induced Nrf2 nucleus translocation, showing that Sul-121 can indeed reduce CSE-induced cellular oxidative stress.

Currently, there are two main categories of anti-oxidative strategies. The first are anti-oxidants which target and neutralize the oxidative stress directly. The second are pharmacological agents known to increase the endogenous antioxidant level for example by increasing Nrf2 activity (Rahman 2006; Kirkham and Barnes 2013). Based on the data in Chapter 6, we hypothesize that Sul-121 acts as anti-oxidant by directly neutralizing ROS. Indeed, Sul-121 did not alter basal Nrf2 nuclear localization. In support, Sul-121 was able to reduce CSE- and hydrogen peroxide-induced ROS levels in a cell-free environment. Since ROS is playing an important role, antioxidants have been shown to be effective in a number of animal models of lung disease (Haddad et al. 2002; Smith et al. 2002). Similar to our study, a catalytic antioxidant [manganese (III) meso-tetrakis (N,N’-diethyl-1,3-imidazolium-2-yl) porphyrin, (AEOL 10150)] has been shown to reduce airway inflammation and neutrophilia in smoke exposed rats (Smith et al. 2002). In addition, by mimicking the endogenous antioxidant glutathione peroxidase, a selenium-based organic complex has been shown to reduce neutrophil-recruiting mediators, as well as lung neutrophil recruitment in an LPS-induced rat pulmonary inflammation model (Haddad et al. 2002).

Oxidative stress has been implicated in a decrease of lung function (Schünemann et al. 1997; Gramiccioni et al. 2010; Stanojkovic et al. 2011; Moussa et al. 2014). In addition, several studies have indicated that ROS production potentiated the contraction of both skeletal and airway smooth muscle (Samb et al. 2002; Zuo and Clanton 2005). Moreover, oxidative stress was reported to directly increase ASM contractility in airways of both human and animal sources (Katsumata et al. 1990; Hulsmann et al. 1994). Therefore, we hypothesize that the anti-oxidative effects of Sul-121 may also contribute to the inhibition of AHR in vivo (Chapter 6). Interestingly, oxidative stress is one of important causes of steroid resistance in COPD (Barnes 2013). By reduction of histone acetylation, histone deacetylase (HDAC) 2 mediates the action of steroids to switch off pro-inflammatory genes (Adenuga and Rahman 2007; Marwick et al. 2007; Barnes 2013). ROS was reported to induce imbalance of acetylation-deacetylation states of histones by reducing HDAC2 activity, via nitrating tyrosine residues on HDAC2 (Ito et al. 2004; Osoata et al. 2009), and phosphorylation of HDAC2 by ROS-activated phosphoinositide 3-kinase (To et al. 2010). Thus, it would be interesting to study in the future if Sul-121 is able to overcome corticosteroid resistance in experimental models of COPD due to its anti-oxidative properties.

By impairing airway epithelial integrity, LPS has been shown to enhance airway epithelial permeability, thereby contributing to an increased infiltration of neutrophils in the airway lumen in several in vivo models (Chignard and Balloy 2000; Evans et al. 2002; Eutamene et al. 2005). Likewise, CSE has been shown to impair airway epithelial integrity in vitro (Heijink et al.
2012; Oldenburger et al. 2014). Epithelial impairment can be attributed to the peroxidative breakdown of polyunsaturated fatty acids (Rahman and Adcock 2006; Rajendrasozhan et al. 2008). Indeed, we found that the lung MDA levels positively correlated with neutrophils in the BALF induced by LPS in vivo (Chapter 6). Therefore, we hypothesize that the Sul-121 prevents LPS-induced airway neutrophilia by preventing epithelial impairment induced by anti-oxidative stress.

Future Perspectives and Scientific Relevance

COPD is ranked as the fourth leading cause of death in the world (GOLD 2015) and is characterized by persistent airflow limitation and chronic lung inflammation. Since COPD is featured with both chronic inflammation and progressive airflow limitation in the lungs (Soriano and Rodriguez-Roisin 2011; GOLD 2015), the most effective pharmacological therapy is suggested to be the combination of anti-inflammatory and bronchodilatory medications (GOLD 2015). So ideally, a drug possessing both broncho-relaxing and anti-inflammatory properties would yield the most efficient treatment of COPD. In the present study, we pre-screened 4 candidates that could represent a novel treatment for COPD. Based on the pre-screening data, we identified Sul-121 as the most promising candidate possessing both broncho-relaxing and anti-inflammatory properties.

Oxidative stress, either induced by inflammatory cells or by inhaled noxious compounds, is an important player in the pathophysiology of COPD (Domej et al. 2014). Besides COPD, oxidative stress is also believed to be involved in the pathogenesis of asthma (Nadeem et al. 2008; Comhair and Erzurum 2010; Dozor 2010; Zuo et al. 2013). Inflammatory cells recruited to the asthmatic airways can initiate the overproduction of ROS, which in turn activate inflammatory transcription factors (such as NF-κB) (Morgan and Liu 2011). Subsequently, the induced (pro-)inflammatory cytokines and chemokines facilitate the up-regulation of adhesion molecules and the increased release of inflammatory mediators, such as IL-4, IL-6, IL-8, TNF-α in this disease (Zuo et al. 2013; Mittal et al. 2014). ROS was also reported to reduce epithelial cell-cell adhesion, thereby contributing to the infiltration of inflammatory cells, including eosinophils, in airway lumen (Usatyuk et al. 2003; Usatyuk et al. 2013; Muresan et al. 2015). Several studies have indicated that ROS production could potentiate the contraction of in airway smooth muscle (Samb et al. 2002). Moreover, ROS induced oxidative stress has also been shown to facilitate the AHR (Katsumata et al. 1990; Sutcliffe et al. 2012; Berair et al. 2013). We have shown that Sul-121 exerts both anti-inflammatory and ASM relaxing properties in vitro, ex vivo and in vivo. Therefore, we speculate that Sul-121 could similarly act as potential treatment option for asthma by targeting oxidative stress.

Two principle types of ROS, superoxide anion and $H_2O_2$, are mainly produced within the mitochondria (Aon et al. 2003; Zorov et al. 2006; Murphy 2009), which are largely found in the
smooth muscle cells due to their essential role of energy generation in muscle contractility (Hoppeler and Fluck 2003). Dysfunction of mitochondria can indirectly increase cellular ROS production and lead to oxidative stress (Wang et al. 2013; Murphy 2013). Interestingly, AKAP1 (aka AKAP121) is a widely expressed mitochondrial AKAP (Carlucci et al. 2008b), which has been identified as an essential regulator of mitochondrial function by interacting with a multivalent signaling complex localizing PKA on the outer wall of the mitochondria (Papa et al. 2002; Livigni et al. 2006; Carlucci et al. 2008b). A study reported that dislocation of AKAP1 from mitochondria by the synthetic Pmit peptides, that encompass the 15–21 or 10–30 regions of the AKAP121 mitochondrial targeting domain, increased mitochondrial ROS production and triggered the death program in cardiomyocytes (Perrino et al. 2010). Others reported that AKAP1 knockout was associated with remarkable mitochondrial structural abnormalities and increased ROS production in a myocardial infarct mouse model (Schiattarella et al. 2016). Although the precise molecular signaling pathways involved in the increased mitochondrial ROS production in cardiac AKAP1 deficient mice are currently not known, the authors proposed that it is likely attributable to the reduced targeting of PKA and associated complexes on the outer mitochondrial membrane (Schiattarella et al. 2016). Since AKAP1 is widely expressed in different tissues and cell types, including ASM cells (Skroblin et al. 2010; Horvat et al. 2012), it is reasonable to speculate that restoration and/or stabilization of mitochondrial AKAP-PKA (e.g. AKAP1-PKA) interactions could be an effective strategy to prevent oxidative stress in the development of obstructive pulmonary diseases such as COPD and asthma. In this context, it is of interest to note that the stability of AKAP1 seems to be controlled by the ubiquitin-proteasome system (Czachor et al. 2016). In response to hypoxic (low oxygen) conditions in the brain, an E3-ubiquitin ligase was found to bind AKAP1 and to tag it for rapid degradation via the ubiquitin/proteasome pathway (Carlucci et al. 2008a). Several studies have demonstrated that mild to moderate oxidative stress increases the activities of the ubiquitin-proteasome system, thereby increasing protein degradation (Shang et al. 1997; Shang et al. 2001; Zhang et al. 2008; Pickering et al. 2010; Shang and Taylor 2011). Therefore, we speculate that oxidative stress may lead to degradation of AKAP1, thereby provoking a further increase in the cellular ROS production. Since Sul-121 bears the property to reduce cellular oxidative stress by directly neutralizing ROS, we hypothesize that Sul-121 could support the maintenance of normal mitochondrial functions by preventing oxidative stress-induced degradation of AKAP1.
Main Conclusions

Overall, the studies presented in this thesis show that:

- Functional AKAP-PKA interactions are important to prevent the induction of a hypercontractile ASM phenotype, by inhibiting the expression of contractile proteins, such as α-SMA and calponin (Chapter 3).
- AKAP-PKA interactions regulate the expression level of α-SMA and calponin on a post-translational level, in a complex that presumably also involves proteasomes (Chapter 3).
- Interruption of AKAP-PKA interactions induces an increase of proliferative markers in ASM, presumably by lowering the expression of AKAP8 known to regulate the cell cycle (Chapters 3 and 4).
- Markers of a hypercontractile phenotype, such as contractile protein expression, and markers of a hyperproliferative phenotype, such as DNA synthesis and cell cycle proteins, can simultaneously be induced in ASM cells and tissue by a single trigger (Chapter 3).
- Sul-90 and Sul-121 have an anti-inflammatory effect in vitro by inhibiting CSE-induced IL-8 release from ASM cells (Chapter 5).
- Sul-90 and Sul-121 cause ASM relaxation independent of an effect on β2-adrenoceptors (Chapter 5 and 6).
- Based on the in vitro effects, out of four Sul compounds, Sul-121 is the most promising novel candidate for the treatment of COPD (Chapters 2 and 5).
- Sul-121 directly neutralizes ROS induced by LPS, a process being accompanied by decreased nuclear translocation of Nrf2 (Chapter 6).
- By directly targeting oxidative stress, Sul-121 reduces activation of NF-κB, thereby preventing IL-8 release and subsequent airway neutrophilia in LPS-treated guinea pigs (Chapter 6).
- Sul-121 prevents LPS-induced AHR in a guinea pig model of COPD, which is not due to its bronchorelaxing effect, but likely caused by inhibiting the LPS-induced lung inflammation (Chapter 6).
- Based on its anti-oxidative, anti-inflammatory and bronchorelaxing effects Sul-121 may represent a novel compound for the treatment of COPD (Chapter 6).
References


Chapter 7


