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EDITORIAL

On Ca^{2+} sensitivity and the airways: not just any smooth muscle

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One of the more difficult questions raised by our students is: ''What purpose does bronchoconstriction serve? What is its evolutionary advantage?'' The most common answer is that narrowing of the airways increases air velocity and shear, thus facilitating removal of materials in the lumen during cough. From a simplistic point of view, it is difficult to see how under other circumstances sustained bronchoconstriction can be anything but detrimental. This differs from vascular smooth muscle (VSM), where sustained vasoconstriction regulates tissue blood flow. Therefore, it should not be surprising if there are qualitative differences in the mechanisms underlying constriction in smooth muscles subserving different functions. In many cases, application of findings in VSM to the airways may not be appropriate, and could be misleading [1]. Considering the prevalence and importance of asthma, however, the number of studies on the contractile mechanisms of airway smooth muscle (ASM) is far fewer than for VSM.

One area where investigations in ASM lag behind those in VSM concerns the control of Ca^{2+} sensitivity of the contractile apparatus. This is despite its potential relevance to asthma, as increased Ca^{2+} sensitivity may at least partly underlie hyperresponsiveness [2–4]. Various factors affect the relationship between intracellular $[Ca^{2+}]$ ($[Ca^{2+}]_i$) and force generation, including modulation of mechanical plasticity [5], but within the contractile apparatus the major determinant is the balance between myosin light chain kinase (MLCK)-dependent phosphorylation of myosin light chain (MLC), and its dephosphorylation by MLC phosphatase (MLCP). It is currently believed that MLCP is primarily regulated *via* Rho kinase-mediated phosphorylation of its targeting sub-unit MYPT1, and protein kinase C (PKC)- and/or Rho kinase-mediated phosphorylation of the MLCP inhibitor CPI-17; both inhibit MLCP activity and increase Ca^{2+} sensitivity [4]. Agonists increase Ca^{2+} sensitivity *via* G-protein coupled receptor activation of the monomeric G-protein RhoA, which activates Rho kinase, or activation of phospholipase C, which activates PKC *via* diacylglycerol. There is also evidence that elevation of $[Ca^{2+}]_i$ can activate RhoA/Rho kinase in both ASM [6] and VSM [7]. An important therapeutic consideration is that bronchodilators that raise cyclic adenosine monophosphate (cAMP; *e.g.* β-adrenoceptor agonists) or cyclic guanosine monophosphate (cGMP; *e.g.* nitric oxide) not only reduce $[Ca^{2+}]_i$, but also Ca^{2+} sensitivity [4]. What is not clear is the relative importance of Ca^{2+} elevating and sensitising pathways for bronchoconstriction in general and asthma in particular, although they are clearly interdependent. In addition, does the regulation of Ca^{2+} sensitivity differ in ASM compared with VSM, the source of the majority of our current knowledge in this area?

Although the role of Ca^{2+} sensitisation and the RhoA/Rho kinase pathway in ASM has been the subject of several papers in recent years, most have relied upon the use of pharmacological inhibitors, and have not directly examined the events leading from receptor activation to enhanced MLC phosphorylation. Therefore, the study by LIU *et al.* [8] in the current issue of the *European Respiratory Journal* is a welcome addition to the literature, although it raises some intriguing questions.

LIU *et al.* [8] examined the time course of tension development and the RhoA/Rho kinase signalling pathway in bovine trachealis, following sub-maximal cholinergic stimulation. Whilst they show that activation of RhoA, Rho kinase, MYPT1 phosphorylation and constriction were sequential as expected, with RhoA activation reaching a peak after ~1 min but tension still increasing at 5 min [8], after 5 min there was a disassociation of tension from RhoA/Rho kinase activity, as tension continued to increase over the next 10 min whilst RhoA and Rho kinase activity declined by ~>50%. This differs from VSM, where agonist-induced RhoA activation is reported to be sustained and parallels force [9]. This raises the question as to why RhoA activity declines in ASM but not VSM. The sustained constriction in the face of reduced RhoA/Rho kinase activity is reminiscent of the ''latch state'', where even though MLC phosphorylation and $[Ca^{2+}]_i$ may fall, tension can be maintained [10]. More recent studies have proposed that this is related to the mechanical plasticity of ASM [5], and a RhoA and RhoA/Rho kinase-mediated series to parallel fibre transition [11]. Whether such mechanisms might be involved here cannot be determined, as neither $[Ca^{2+}]_i$ nor MLC phosphorylation were examined.

BAI and SANDERSON [12] have also recently presented data concerning Ca^{2+} sensitisation in ASM, but using a very different model: precision-cut slices of murine lung. $[Ca^{2+}]_i$ was effectively clamped by treatment with ryanodine and caffeine, and could be regulated by altering extracellular Ca^{2+}. Whereas increasing $[Ca^{2+}]_i$ caused sustained constriction in pulmonary arteries, the airways showed only a transient constriction that returned towards baseline within minutes, implying loss of Ca^{2+} sensitivity. Subsequent cholinergic

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stimulation caused a sustained Rho kinase- and PKC-dependent airway constriction without change in $[Ca^{2+}]_i$ [12]. There are intriguing parallels with the study of LIU *et al.* [8]. Whereas BAI and SANDERSON [12] demonstrated a fall in ASM Ca^{2+} sensitivity over time following the elevation of $[Ca^{2+}]_i$, LIU *et al.* [8] demonstrated a fall in RhoA/Rho kinase activity over time following agonist stimulation, which would also be associated with elevation of $[Ca^{2+}]_i$; neither apparently occur in VSM [9, 12]. Although it is tempting to speculate that these phenomena are related, and reflect transient activation of the Rho/Rho kinase pathway by elevation of $[Ca^{2+}]_i$ coupled with a sustained component activated by agonist, the methodological differences and lack of directly comparable measurements (*e.g.* $[Ca^{2+}]_i$, biochemical measurements) make it impossible to draw any firm conclusion.

Nevertheless, both studies reaffirm that RhoA/Rho kinase-mediated Ca^{2+} sensitisation is important for sustained constriction of ASM induced by cholinergic stimulation [8, 12]. The studies also examined the action of bronchodilators on Ca^{2+} sensitisation. In the model of BAI and SANDERSON [12], where $[Ca^{2+}]_i$ is clamped, agents that raise cAMP (forskolin, phosphodiesterase inhibitors) caused significant ASM relaxation that must perforce be due to Ca^{2+} desensitisation. Conversely, LIU *et al.* [8] showed that elevation of either cAMP (isoprenaline, salmeterol) or cGMP (NO-donor *S*-nitroso-*N*-acetyl penicillamine) had relatively small effects on RhoA or Rho kinase activity (~20% and 30–40% suppression, respectively), compared with the ~75% suppression of MLCK activity and 90% suppression of tension. This suggests that here, where $[Ca^{2+}]_i$ was free to change, the large majority of the relaxation was associated with a fall in $[Ca^{2+}]_i$; in fact, this might also account for the reduction in Rho kinase activity, as the latter is activated by Ca^{2+} in ASM [6].

Another recent study by OGUMA *et al.* [13], in guinea pig trachealis, may provide additional insight. In this study, isoprenaline and cAMP-induced Ca^{2+} desensitisation was independent of either Rho kinase or PKC, but was still dependent on MLCP. A prime suspect for this effect is telokin, a 17-kDa protein that is specific for smooth muscle, phosphorylated by both cAMP and cGMP, and promotes Ca^{2+} desensitisation *via* direct activation of MLCP [14, 15]. Telokin-deficient mice demonstrate greater Ca^{2+} sensitivity and reduced cGMP-induced Ca^{2+} desensitisation in intestinal smooth muscle, but interestingly there was no effect on the Ca^{2+} sensitivity of aortic smooth muscle, which normally has five-fold less expression of telokin than intestinal smooth muscle [16]. So far, the expression and function of telokin has not been examined in ASM.

Whilst the studies described above provide important information about the role of Ca^{2+} sensitisation in ASM and its modulation by bronchodilators, differences in methodologies, tissues and species limit their combined impact. However, biochemical measurements, such as those of LIU *et al.* [8], require the tissue mass provided by trachealis or large bronchus; they would be virtually impossible in the lung-slice preparation of BAI and SANDERSON [12], which is otherwise excellent for examining bronchiole and pulmonary artery function *in situ*. Nevertheless, it is known that the ASM of trachealis and large bronchus differs in several respects from that of functionally important small bronchioles, including ion channels and Ca^{2+} signalling [17, 18]. In a murine model of allergic asthma, only bronchial but not tracheal ASM exhibited hyperresponsiveness [19]. There may also be differences in mechanisms associated with Ca^{2+} sensitisation. Species differences are recognised, and for direct relevance to asthma, ideally all studies should be performed in human small bronchioles. Although these are possible, we know to our cost that they are difficult, availability of suitable tissue is very limited, and most such tissue is derived from lung resections from older patients with tumours [17, 20].

In summary, knowledge of the mechanisms underlying and modulating Ca^{2+} sensitivity of airway smooth muscle may be of significant importance to our understanding of the pathophysiology of asthma, and the function of bronchodilators. Therefore, the study of LIU *et al.* [8] is very welcome, but for the reasons discussed previously is necessarily limited. Several key aspects of all the studies discussed need to be further investigated, including whether they can be translated to (human) bronchioles, the mechanisms underlying the time-dependent fall in agonist-induced RhoA/Rho kinase activity, and Ca^{2+}-induced tension in airway smooth muscle and, in particular, the potential role of telokin in the regulation of airway smooth muscle Ca^{2+} sensitivity. However, one thing is sure; we cannot rely on studies in other smooth muscles if we want to understand the airways.

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