Smooth muscle: PKC-induced Ca^{2+} sensitisation by myosin phosphatase inhibition

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When arterial smooth muscles are stimulated with certain agonists such as phenylephrine contractile force increases while the intracellular free Ca^{2+} concentration may increase very little or even not at all (cf. Bradley & Morgan, 1987). In this case, force may be enhanced by raising the responsiveness of the contractile machinery or – for that matter – the sensitivity of the myofilaments to intracellular free Ca^{2+} (some 100 nM under resting conditions). The nature of mechanisms underlying Ca^{2+} sensitisation in pharmacomechanical coupling has been shown to involve various quite different intracellular signalling pathways and signalling molecules, such as small G-proteins (e.g. Rho A) and enzymes such as Rho-associated kinase, MAP kinase and various isoforms of protein kinase C (PKC) (cf. Somlyo & Somlyo, 1994; for review see Arner & Pfitzer, 1999). Based on the observation that phorbol esters, which are well known activators of PKC, cause a sustained contraction of arterial smooth muscle (Rasmussen *et al.* 1984) and also increase the sensitivity of the contractile apparatus to added Ca^{2+} in permeabilised smooth muscle preparations (Chatterjee & Tejeda, 1986), a role for PKC-induced Ca^{2+} sensitisation in tonic smooth muscle contraction was proposed long ago. During smooth muscle activation, PKC has been shown to be translocated from the cytosol to the cell membrane (Khalil *et al.* 1992), where it may be activated by diacylglycerol (DAG). It then initiates a signal sequence leading to the phosphorylation of specific intracellular receptors called receptors for activated C-kinase (RACKs). Thus the question arises as to the nature of RACKs involved in Ca^{2+} sensitisation of the myofilamets by PKC.

The primary mechanism of smooth muscle contractile activation is the phosphorylation of myosin light chain (MLC) though, admittedly, the thin filament proteins caldesmon and calponin may also play a role (Arner & Pfitzer, 1999). It is now well established that the physiological increase in the phosphorylation of MLC is caused not only via Ca^{2+}/calmodulin activation of myosin light chain kinase but also by a decrease in the dephosphorylating enzyme activity (e.g. Kitazawa *et al.* 1991). In a paper appearing in this issue of *The Journal of Physiology*, Kitazawa *et al.* (1999) now show that in permeabilised (skinned) preparations of arterial smooth muscle, PKC enhances contractile force at a given submaximal concentration of free Ca^{2+} by increasing the level of MLC phosphorylation via inhibition of myosin phosphatase. This inhibition is mediated by phosphorylation of a novel RACK called CPI-17. This smooth muscle specific myosin phosphatase inhibitor exerts its phosphatase inhibitory and hence Ca^{2+} sensitising action in permeabilised preparations only when phosphorylated by PKC. These discoveries were made by using a differential skinning method: while intact arterial smooth muscle or preparations permeabilised by *Staphylococcus aureus* α-toxin or with the saponin ester β-escin became sensitised to Ca^{2+} by phorbol esters and other activators of PKC, fibres demembranated with the detergent Triton X-100 did not. The reason was that Triton-skinned fibres had lost the smooth muscle specific phosphatase-1 inhibitor protein CPI-17 and most of PKC. However, reconstitution of these fibres with PKC and CPI-17 increased MLC phosphorylation and the response to Ca^{2+} suggesting that, indeed, this kinase and the phosphatase inhibitor were the key players, causing increased myosin phosphorylation and contraction at submaximal Ca^{2+} concentration.

These important new findings challenge current ideas (cf. Singer, 1996) suggesting that the physiological activation of PKC in vascular smooth muscle and its sensitisation to Ca^{2+} are mediated by mechanisms involving mitogen activated protein kinase-dependent caldesmon phosphorylation rather than phosphorylation of MLC by PKC. Now, the question arises as to what extent the proposed mechanisms may be operating in smooth muscle *in vivo*. It seems quite puzzling how in intact cells PKC can phosphorylate CPI-17 which, apparently, is located in the cytosol, while PKC activation requires its translocation to the cell membrane. Perhaps, CPI-17 shuttles between membrane-bound active PKC and thick filament-bound myosin phosphatase. Alternatively, membrane-bound (activated) PKC may be in rapid equilibrium with cytosolic PKC. In the cytosolic compartment the quantity of activated PKC, though low, may then be still sufficient to cause the Ca^{2+} sensitising downstream effect mediated by the phosphorylation of the smooth muscle specific phosphatase-1 inhibitor CPI-17 targeted to the thick filaments.

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