Growth-dependent modulation of receptor-effector coupling for intracellular Ca^{2+} (Ca^{2+}_i) handling in vascular smooth muscle cells (VSMCs)

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Vascular smooth muscle cells (VSMCs) modify the phenotype expression both *in vitro* and *in vivo*, as might be important for the pathogenesis of atherosclerosis. Previously, we have reported that Ca^{2+}_i responses in VSMCs are heterogeneous in agonist stimulation (high K^+ depolarization, caffeine and angiotensin (I) and dependent on the cell growth (Shin *et al.*, 1991, *Circ. Res.* **69**, 551–6) (Masuo *et al.*, 1991, *Circ. Res.* **69**, 1327–36). As is similar to most vasoactive peptides, endothelin-1 (ET_1) employs two signal transduction systems; (I) receptor to transient Ca^{2+} release from the sarcoplasmic reticulum and (II) receptor to sustained Ca^{2+} influx through voltage-dependent Ca^{2+} channels in the sarcolemma. Using 2-dimensional Ca^{2+}_i image analysis (Shin *et al.*, 1992, *J. Biol. Chem.* **267**, 20377–82) of cloned VSMCs (A7r5), we have identified two cell populations showing both transient and subsequent sustained phases or the sustained phase alone after sufficient dose (100 nM) of endothelin-1 (ET_1) stimulation. To elucidate how ET_1 selects two Ca^{2+} signalling pathways, we stopped or promoted the cell growth in serum-free medium or by PDGF and found that lack of the transient response is caused not by a defect in ET_1-receptors or effectors (IP_3-sensitive Ca^{2+} channels or voltage-dependent Ca^{2+} channels), but by uncoupling between the ET_1-receptor and effector during cell growth. If time is available, we will present evidence that the heterogeneity of VSMCs were histologically confirmed *in vivo*; intimal hyperplasia induced by endothelial denudation in rat carotid artery, which is an animal model of human arteriosclerosis.

Peptide mimetics as a probe of the contractile cycle

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The various sites by which actin and myosin contact each other during the contractile cycle have been identified using synthesized peptides to mimic each individual site in turn and employing the partner protein to act as the molecular template. Specific binding has been established by using NMR techniques, by showing they were competitive inhibitors of the acto-S1 Mg.ATPase activity and by direct binding studies. The structure of each peptide in solution has been investigated by 2D-NMR techniques and was found to possess the same elements of secondary structure as in the parent protein. In some cases, the structure of the peptide bound to its partner has been determined by the transferred NOESY experiment which revealed subtle changes in the peptide structure on binding, suggesting how the allosteric response between different sites might be communicated. The sites on actin that have been identified as binding to S1 are contained within the N-terminal region, residues 338–349, 77–94 and the loop around residue 40. The sites on S1 identified as containing residues binding to actin are 400–416, 528–552, and 568–579. Experiments monitoring the displacement of S1 from actin (± ATP) have allowed contacts that occur in the weakly attached acto-S1 complex to be distinguished from the strongly attached states and have suggested an ordered and sequential docking of the two proteins.

Colocalization of Ca^{2+} transporting proteins and functional Ca^{2+} microdomains in smooth muscle

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Functional intracellular Ca^{2+} microdomains were studied by X-ray microanalysis (EPMA) in isolated urinary bladder myocytes shock-frozen either before or 500 ms after SR Ca^{2+} release induced by depolarization and I_{Ca}. Ca^{2+} release is indicated by less numerous spots of high total [Ca] close to surface membrane while [Ca] spots close to the nucleus did not change significantly. It is concluded that Ca^{2+} release through ryanodine-receptors occurs predominantly at the peripheral SR close to the surface membrane. Three dimensional immuno-fluorescence with antibodies labelled by fluorescein or texas red reveal colocalization of Na^+-Ca^{2+} exchanger, L-type Ca^{2+} channels and ryanodine-sensitive Ca^{2+} release channels within the same voxel ($200 \cdot 200 \cdot 700\ nm^3$) close to the surface membrane. The above Ca^{2+} transporting proteins co-localized with caveolin but not with vinculin suggesting a close spatial relation to the caveolae. Immuno gold labelling localized the $InsP_3$-receptors mostly in the inner regions of the cell.