5. Summary

Mammalian homologues of the Drosophila transient receptor potential (TRP) protein, more specifically TRPC1 – 7, have been proposed to function as receptor-stimulated Ca^{2+} entry channels. To date, the properties of TRPC1 – 7 have been extensively studied in heterologous expression studies. By contrast, relatively little information is available on their role in native tissues. Therefore, the major aim of this study was to evaluate the possible role of TRPC1 – 7 in vasoconstrictor-induced Ca^{2+} entry in rat A7r5 aortic smooth muscle cells.

In A7r5 cells, [Arg^8]-vasopressin (AVP) induced an increase in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) consisting of Ca^{2+} release and Ca^{2+} influx. Whole-cell voltage-clamp recordings revealed the activation of a nonselective cation current with a doubly-rectifying I-V relation strikingly similar to those described for some heterologously-expressed TRPC isoforms. The current was inhibited by lanthanum (La^{3+}) and gadolinium (Gd^{3+}). External Ca^{2+} exerted complex effects, involving both facilitatory and inhibitory mechanisms. Direct activation of G proteins by infusion of aluminium fluoride activated a cation current with properties identical to those of the AVP-induced current. Furthermore, activation of the PLCγ-coupled platelet-derived growth factor receptor also stimulated the current. However, current activation was neither dependent on store depletion nor on increased $[Ca^{2+}]_i$. Since currents identical to those evoked by AVP were activated by application of 1-oleoyl-2-acetyl-sn-glycerol (OAG) in a protein kinase C-independent way, the TRPC3/6/7 subgroup is suggested to be involved in mediating these currents. Like TRPC6-mediated currents, cation currents in A7r5 cells were increased by flufenamate. Northern hybridization revealed mRNA coding for TRPC1 and TRPC6. Hence, TRPC6 is suggested to be a determining molecular component of receptor-stimulated Ca^{2+}-permeable cation channels in A7r5 cells.

To investigate whether TRPC6 plays a more general role in vascular smooth muscle, two other smooth muscle cell preparations were examined. In smooth muscle cells derived from the rat vena cava, AVP stimulated Ca^{2+} influx. However, activation of a corresponding cation current could not be detected, and no mRNA for TRPC2-6 was found in this cell line. Preliminary studies on primary cultures of smooth muscle cells

derived from neonatal and adult rat aorta revealed the activation of a cation current similar to the current observed in A7r5 cells.

To compare the properties of the native currents in A7r5 cells with those mediated by heterologously-expressed TRPC6, rat TRPC6 was cloned and electrophysiologically characterized in recombinant expression studies. Furthermore, the properties of recombinant TRPC6 were compared to those of mouse TRPC5, which belongs to the structurally and functionally distinct TRPC4/5 subgroup of TRPC channels. External Ca^{2+} was found to have an inhibitory effect on currents mediated by rat TRPC6, whereas currents through recombinant mouse TRPC5 were potentiated by increased extracellular Ca^{2+}. Moreover, rat TRPC6 was blocked by external La^{3+} or Gd^{3+}, while whole-cell currents through mouse TRPC5 were reversibly stimulated by micromolar and inhibited by millimolar concentrations of both ions. The dual effects of La^{3+} on mTRPC5 were also reflected on the single-channel level, with increasing La^{3+} concentrations reducing the single-channel conductance, but increasing the open probability. Hence, external Ca^{2+} and micromolar concentrations of La^{3+} and Gd^{3+} have opposite effects on whole-cell currents through recombinant TRPC5 and TRPC6 channels and may be a tool to identify and discriminate the involvement of the TRPC3/6/7 and the TRPC4/5 subgroup in receptor-operated cation conductances of native cells.

The major finding of the present study is that TRPC6 is a molecular component of vasoconstrictor-activated cation channels in A7r5 smooth muscle cells. Importantly, the present study demonstrates that an endogenous receptor-stimulated cation current shows properties identical to those described for TRPC isoforms in heterologous overexpression studies.