Molecular architecture of the sodium–glucose cotransporter

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Sodium–glucose cotransporters are responsible for the accumulation of glucose and galactose in cells ranging from bacteria to man. They belong to a large gene family, the SGLT gene family, where a common function appears to be sodium (or proton) cotransport of substrates such as sugars, ions, amino acids and vitamins. We have previously proposed a cotransport model where ion–substrate cotransport occurs by ligand- and voltage-induced conformation changes, and where the rate and direction of transport is a function of voltage and the concentration of the ligands on each side of the membrane. Our recent studies to test this model have focused on the structure and function of two family members, the human and *Vibrio paraheamolyticus* Na^+–glucose and galactose cotransporters, hSGLT1 and vSGLT. Both proteins have been expressed in *E. coli* and *Xenopus laevis* oocytes for functional studies and both have been purified to homogeneity from *E. coli* and reconstituted into liposomes for structural studies using electron microscopic and spectroscopic methods (Turk *et al.* 2000; Quick & Wright, 2002). Oocytes were harvested from frogs anaesthetized with Tricaine in accordance with UCLA and NIH animal welfare guidelines. In this presentation I will summarize our data showing ligand-induced global changes in conformation of vSGLT in proteoliposomes using attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy and H/D exchange (Le Coutre *et al.* 2002), and ligand- and voltage-induced local changes in conformation in proteoliposomes and oocytes using extrinsic fluorescence probes covalently attached to designated cysteine mutants (Meinild *et al.* 2002). Freeze fracture electron microscopy demonstrated that both the functional cotransporters are monomers. ATR-FTIR reveals stepwise increases in helical content of vSGLT upon binding of sodium and D-galactose, and this is accompanied by stepwise reductions in H/D exchange. These experiments indicate discrete conformational changes in the protein during the catalytic transport cycle of the protein. Fluorescence studies of both vSGLT and hSGLT1 show that the conformational changes involve specific domain in helices 10, 11 and 12 and the extracellular linker between helices 10 and 11. These results support our kinetic model for transport and they are incorporated into a structural model.

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Structure/function relationship in type IIa Na–P_i cotransporters: transport and regulation

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Renal proximal tubular and small intestinal P_i-absorption/reabsorption are Na^+ coupled and secondary active. Thereby, transport across the brush-border membrane is rate limiting and involves physiologically regulated Na^+–P_i cotransporters. In the small intestine regulatory mechanisms require hours/days (e.g. P_i-diet, 1.25 $(OH)_2$ Vit D3); in the renal proximal tubule regulation occurs within minutes (parathyroid hormone, other peptide hormones; Pi-diet) as well as after hours (P_i-demand; P_i-diet). These regulatory phenomena are associated with altered brush border membrane expression of Na^+–P_i cotransporters.

Brush border membrane P_i-flux is mediated by type II Na^+–P_i cotransporters: type IIa (and to a smaller extent type IIc) in the kidney, type IIb in the small intestine. They mediate a 3 Na^+ to 1 P_i cotransport: an interaction with 1 Na^+ ion is followed by interaction with 1 P_i and then by 2 additional Na^+ ions. In the absence of P_i transfer of Na^+ can occur (slippage).

Type II cotransporters contain at least eight transmembrane domains (TM) and cytoplasmic oriented NH_2- and COOH-termini. Detailed structure/function relationships have been performed on the type IIa isoform: a glycosylated large extracellular loop separates the transporter into two domains (TM1–3 and TM4–8), both required for transport function. Predicted intracellular loop 1 (ICl-1) and extracellular loop 3 (ECL-3) contain intrasequence homologies. Chimera studies (IIa and IIb) led to the identification of sites contributing to Na^+ affinity (TM-5) or pH sensitivity (ECL-3). Cysteine-scanning studies identified important (α-helical) structures in ICl-1 and ECL-3, suggesting that they are components of a permeation pore participating in slippage and cotransport. The type IIa cotransporter exerts its function as a monomer.

Altered brush-border membrane expression is achieved by specific membrane retrieval/insertion processes. Membrane retrieval of type IIa has been extensively characterized by taking parathyroid hormone (PTH) as an example. PTH activates protein kinase A and C regulatory cascades: luminal PTH preferentially activates the kinase C and basolateral PTH the kinase A regulatory pathway; thereby an activation of MAPK-kinase seems to play a central role in the internalization. At the level of the transporter, specificity for internalization is given by two basic amino acid residues in ICL-3. Reinsertion of type IIa Na^+–P_i cotransporters depends on sequences contained in the COOH-terminus: a terminal TRL-sequence and an internal pair of basic amino acid residues; for the type IIb cotransporter a COOH-terminal L-residue determines apical expression. The terminal TRL-residues are probably required for apical scaffolding of the type IIa cotransporter involving PDZ-modules of NaP_i-Cap1 (4 modules) and NHE-RF1 (2 modules). These proteins interact at one of the PDZ-modules with the type IIa cotransporter and permit via an interaction at the remaining sites to build apical complexes containing other brush-border membrane proteins and/or elements of the cellular regulatory machinery (e.g. D-AKAP2; dual specific kinase A anchoring protein).

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Antibody-based targeted proteomic analysis of renal tubule sodium transporter regulation: role of angiotensin II

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Complementary DNAs for each of the major sodium ion transporters and channels expressed along the renal tubule have been cloned over the past 15 years. The consequence is the generation of a broad range of cDNA and antibody probes that can be used to investigate physiological mechanisms on a molecular level. An ensemble of such probes can be exploited for comprehensive analysis of integrative physiological processes, approaches which are referred to as 'physiological genomics' or 'physiological proteomics'. The current studies utilize a targeted proteomic approach to allow comprehensive analysis of sodium transporter and water channel protein abundance along the renal tubule, using an ensemble of rabbit polyclonal antibodies for semiquantitative immunoblotting and immunocytochemistry of renal homogenates (Masilamani *et al.* 1999; Brooks *et al.* 2001; Knepper & Masilamani, 2001; Wang *et al.* 2001). The strategy is to profile the transporter abundance changes along the nephron in response to known regulators of renal sodium excretion (aldosterone, angiotensin II, noradrenaline, vasopressin, endothelins, NO, cyclo-oxygenase products, atrial natriuretic factor, etc.), and then to look for the same patterns of responses in pathophysiological models of abnormal regulation of blood pressure (Dahl salt-senstive rat, 2-kidney, 1-clip Goldblatt rat) or extracellular fluid volume (congestive heart failure, cirrhosis, nephrotic syndrome). By this approach we can generate hypotheses regarding the mediators of abnormal sodium balance in pathophysiological models of human disease.

Current studies are focusing on regulatory targets for angiotensin II. These studies are employing renal tubule Na transporter profiling in three experimental models: (1) angiotensin II receptor AT_{1a} knockout mice; (2) rats infused with the AT_1 receptor blocker candesartan; and (3) rats infused with angiotensin II. The studies point to several potential molecular targets for regulation: (a) NaP_i-2 (sodium phosphate cotransporter type II in proximal tubule): marked decrease in abundance in AT_{1a} knockouts and with AT_1 receptor blockade. (b) NBC1 (sodium bicarbonate cotransporter in proximal tubule): marked decrease in abundance with AT_1 receptor blocked; increased abundance with angiotensin II infusion. (c) α-ENaC (α subunit of epithelial sodium channel): decreased in AT_{1a} knockout mice and with AT_{1a} receptor blockade; increased with angiotensin II infusion. (d) β- and γ-ENaC: increased in AT_{1a} knockout mice and with AT_{1a} receptor blockade. There were no consistently detectable changes in the abundances of NHE3 or NKCC2 in any of these protocols. Further studies focusing on the ENaC changes in response to candesartan demonstrated that the changes can occur in the presence of mineralocortocoid receptor blockade with spironolactone and without substantial decreases in circulating aldosterone. These findings therefore suggest the possibility that regulation of ENaC subunit abundances by angiotensin II is due a direct action of the peptide on the collecting duct cells rather than a response to mineralocorticoid.

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$P2X_7$ receptors and downstream signalling

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P2X receptors are ATP-gated ion channels in the plasma membrane, but activation of the $P2X_7$ receptor couples cytoskeletal rearrangements such as membrane blebbing. We used affinity purification of the rat $P2X_7$ receptor followed by mass spectroscopy and immunoblotting to identify proteins in human embryonic kidney cells that interact with the receptor. We found laminin α_3, integrin β_2, β-actin, α-actinin, supervillin, MAGuK, three heat shock proteins, phosphatidylinositol 4-kinase, and the receptor protein tyrosine phosphatase β. Activation of the $P2X_7$ receptor with the agonist 2′,3′-benzoyl-(4-benzoyl)ATP resulted in its dephosphorylation on tyrosine; by systematic mutagenesis we identified the residue involved as Tyr^{343} in the putative second transmembrane domain. Whole-cell recordings from cells expressing $P2X_7$ receptors showed that repeated applications of a high concentration of agonist led to a strong decline in the amplitude of the current; this was prevented by phosphatase inhibitors. Phosphatase inhibitors also accelerated membrane blebbing. The results indicate that activation of the $P2X_7$ receptor results in the stimulation of an associated receptor protein tyrosine phosphatase. Dephosphorylation of the receptor on Tyr^{343} inhibits the flow of ionic current and impairs coupling to the downstream effectors leading to the cytoskeleton.

Diverse roles of CLC chloride channels: lessons from disease in mice and man

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CLC Cl^- channels form an evolutionarily old gene family that has nine members in mammals. While the first branch of this family encodes plasma membrane channels, it is now clear that channels belonging to the other two branches reside primarily in intracellular organelles.

ClC-Ka and ClC-Kb (both belonging to the first branch) are expressed in different nephron segments. Mutations in ClC-Kb underlie Bartter syndrome III, while the disruption of ClC-Ka in mice caused diabetes insipidus. While this indicated a role in transepithelial transport, both proteins did not yield currents upon heterologous expression. It is now clear that they need barttin, a protein with two transmembrane spans, for functional expression (Estévez *et al.* 2001). Mutations in barttin underlie Bartter syndrome type IV, which combines severe renal salt loss with deafness. Indeed, ClC-K/barttin heteromers are additionally involved in K^+ secretion across the stria vascularis of the inner ear by recycling Cl^- taken up by a Na^+–K^+–$2Cl^-$ cotransporter (Estévez *et al.* 2001).

It has long been known that Cl^- conductances provide an electrical shunt that is needed for the efficient operation of the H^+-ATPase that acidifies vesicles of the endocytotic and secretory pathways. However, the molecular identities of the underlying Cl^- channels have remained obscure until such a role was recognised for ClC-5. This channel is mutated in Dent's disease, a disorder characterised by low molecular weight proteinuria and kidney stones (Lloyd *et al.* 1996). ClC-5 resides in endosomes of the proximal tubule (PT), where it co-localises with the H^+-

ATPase and endocytosed proteins (Günther *et al.* 1998). This suggested a role in the acidification of the endocytotic pathway. Disrupting ClC-5 in mice affects both fluid-phase and receptor-mediated endocytosis, as well as the endocytotic retrieval of certain plasma membrane proteins in the PT (Piwon *et al.* 2000). As the PT endocytoses hormones such as PTH and 25(OH)VitD3, this leads to changes in calcio-tropic hormone levels and to secondary changes in the renal handling of phosphate and calcium. Thus the vesicular ClC-5 Cl^- channel is crucial for endocytosis.

We have also disrupted the highly homologous ClC-3 Cl^- channel that is expressed in brain and several other organs (Stobrawa *et al.* 2001). This led to a nearly complete degeneration of the hippocampus and photoreceptors. ClC-3 was localised to late endosomes and synaptic vesicles, to whose acidification it contributes. The degeneration of the hippocampus may be due to an altered filling of synaptic vesicles (which depends on the electrochemical H^+ gradient), or to altered intracellular trafficking.

Finally, we have disrupted ClC-7, a broadly expressed member of the third branch of the CLC family (Kornak *et al.* 2001). This led to severe osteopetrosis, which is due to a failure of osteoclasts to acidify the resorption lacuna. ClC-7 is normally present in late endosomal to lysosomal compartments, but is inserted together with the H^+-ATPase into the osteoclast ruffled border upon attachment to bone. Stimulated by this finding, we also demonstrated that human patients with severe osteopetrosis have mutations in either the ClC-7 Cl^- channel (Kornak *et al.* 2001), or in a subunit of the H^+-ATPase (Kornak *et al.* 2000).

Thus the interplay between the H^+-ATPase and various CLC Cl^- channels has diverse and important roles for the cell and the organism.

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Lipid and mechano-gated 2P domain potassium channels

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Potassium channels form the most diverse family of ion channels with more than 70 genes cloned in humans. They can be broadly subdivided into three structural classes made of two, four or six transmembrane segments (TMS) (Patel & Honoré, 2001*a*). All potassium channels can be recognized by the presence of a conserved motif called the P domain (the pore-forming region), which is part of the potassium conduction pathway. The two TMS and six TMS classes contain a single P domain while the class of four TMS subunits contains two P domains in tandem. Functional potassium channels are tetramers of pore-forming subunits for the two and six TMS classes and probably dimers in the case of the four TMS class.

The family of mammalian 4TMS/2P potassium channel subunits has increased to 14 members (Patel & Honoré, 2001*a*). These subunits share the same structural motif but low sequence identity is found outside the P domains. TREK-1 (KCNK2), TREK-2 (KCNK10) and TRAAK (KCNK4) subunits form a structural as well as a functional subgroup. Human TREK channels are highly expressed in the central and peripheral nervous systems, but are absent from the heart. TREK channels are opened by a variety of physical and chemical stimuli (Patel & Honoré, 2001*a*).

TREK channel activity is elicited by increasing mechanical pressure applied to the cell membrane (Patel & Honoré, 2001*a*; Patel *et al.* 2001). At the whole cell level, TREK-1 is modulated by cellular volume. Intracellular acidosis converts TREK mechano-gated channels into constitutively active channels. Finally, heat gradually and reversibly opens TREK-1 with an exceptional Q_{10} value of 7. Deletional analysis demonstrates that the carboxy terminus, but not the amino terminus and the extracellular M1P1 loop, is critical for activation of TREK-1 by stretch, intracellular acidosis and heat.

TREK channels are reversibly opened by anionic polyunsaturated fatty acids including arachidonic acid. The activation is either direct on the channel protein or via a membrane effect (Patel *et al.* 2001). Lysophospholipids containing large polar heads are additional potent openers. TREK channels are the target for volatile general anaesthetics including halothane and isoflurane (Patel & Honoré, 2001*b*). Opening of these channels by inhalational anaesthetics induces cell hyperpolarization and may contribute to general anaesthesia (Patel & Honoré, 2001*b*). Chemical activation of TREK-1 is also critically dependent on the carboxy terminal domain (Patel & Honoré, 2001*a, b*; Patel *et al.* 2001).

TREK channels are additionally regulated by the neuro-transmitter/cAMP/PKA pathway (Patel & Honoré, 2001*a*). For instance, opening of TREK-1 by lipids is reversed by protein kinase A stimulation. Protein kinase A-mediated phosphorylation of Ser333 in the carboxy terminus mediates TREK-1 closing. TREK-1 activity is also inhibited by the protein kinase C pathway, although the phosphorylation site remains to be identified.

The biophysical and pharmacological properties of TREK channels resemble those of the *Aplysia* S-type potassium channel (Belardetti & Siegelbaum, 1988; Patel & Honoré, 2001*a*). The S channel is responsible for the control of presynaptic facilitation of transmitter release that underlies behavioural sensitization, a simple form of learning and memory. Considering that TREK channels are highly expressed in the hippocampus and the cerebral cortex, they may play a significant role in cognition (Patel & Honoré, 2001*a*).

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Structure–function relationships in potassium channels

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Discovered recently, K2P potassium channels (encoded by KCNK genes) are numerous, widespread and notable for their unique structure: each subunit possesses two pore-forming P domains. The channels are also remarkable in function, serving as highly regulated, potassium-selective leaks. Fundamental to the excitability of nerves and muscles, the molecular basis for leak currents had previously been a mystery. Channels in-hand,

we can now consider why they remained hidden and the structural basis for their operation. Many of our most basic questions remain to be answered.

K_{ATP} channels: physiological surprises

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K^+ channels that are closed by ATP (K_{ATP}) were discovered by Aki Noma in the heart, and subsequently reported in many other cell types, including pancreatic β-cells. A paradigm for control of insulin secretion is that glucose metabolism elevates cytoplasmic [ATP]/[ADP] in pancreatic β-cells, closing K_{ATP} channels, and causing depolarization, Ca^{2+} entry, and insulin release. Sulfonylureas, which inhibit K_{ATP} channel activity, are in major clinical use for enhancing insulin secretion in diabetic patients, and persistent hypersecretion of insulin is seen in patients with mutations that decrease K_{ATP} activity. In the heart there is evidence for involvement of K_{ATP} in action potential (AP) shortening and response to ischaemia, but there remains no consensus of when, and where, cardiac K_{ATP} channels are active.

Susumu Seino, Joe Bryan and colleagues cloned genes encoding K_{ATP} in the mid-1990s; the channels are formed of pore-forming Kir6.2 subunits associated with regulatory sulfonylurea receptors (SUR1 in the pancreas, SUR2A in the heart). Each gene has been 'knocked-out' by recombinant approaches; knock-out of Kir6.2 or SUR1 impairs insulin secretion and abolishes glucose dependence, but neither fully reiterates the maintained hyperinsulinaemia that is observed in human HI patients. Knock-out of Kir6.2 or SUR2 genes abolishes AP shortening in myocardial ischaemia but does not give an indication of when the channels are likely to activate normally.

As an alternative approach to probing K_{ATP} function, we are examining the consequences of transgenic expression of mutant channels. Decreased ATP sensitivity is predicted to cause decreased insulin secretion and perhaps diabetes in the pancreas, and to cause AP shortening, and perhaps inexcitability in the heart. We generated transgenic mice expressing mutant Kir6.2 subunits, in pancreatic β-cells under insulin gene promoter (RIP1) control in β-cells, and under α-myosin heavy chain (MHC) promoter control in heart. The constructs we used were Kir6.2[DN] and Kir6.2[DN,K185Q], which generate K_{ATP} channels that are respectively ~10-fold and ~300-fold less sensitive to ATP inhibition when expressed with SUR1 subunits in COSm6 cells.

Under RIP1-control, we obtained no Kir6.2[DN,K185Q] founders, but five founders expressing the Kir6.2[DN] construct. F1 mice all showed the same dramatic phenotype: they developed severe hyperglycaemia, hypoinsulinaemia and ketoacidosis within 2 days, and typically died within 5 days. Islet morphology, insulin localization, and α- and β-cell distributions were normal (< day 3), pointing to reduced insulin secretion as the single causal mechanism. The data indicate that normal K_{ATP} channel activity is critical for maintenance of euglycaemia, and that even very minimal overactivity may cause diabetes by inhibiting insulin secretion and in accordance, Matthias Schwanstecher and colleagues have recently reported that even a 2-fold reduction of ATP sensitivity resulting from the E23K mutation in human Kir6.2 may be causal in Type II diabetes.

In contrast, viable founder mice expressing both Kir6.2[DN,K185Q]-GFP and Kir6.2[DN]-GFP in the heart were obtained. K_{ATP} channels from Kir6.2[DN,K185Q]-GFP transgenic myocytes exhibit a spectrum of ATP sensitivities, due to heteromultimerizations of endogenous and transgenic subunits, but are on average very ATP insensitive. In the highest expressing (line 4) myocytes, ATP sensitivity is reduced almost two orders of magnitude from ~50 mM to about 4 mM! Many studies indicate that given the high density of K_{ATP} conductance in heart cells, only ~1% of maximal channel activity would be necessary to cause 50% shortening of the AP. Severe AP shortening would thus be expected in these transgenic mice, but none is observed, either in isolated cells or intact hearts. The immediate reason is clear enough – despite being very ATP-insensitive, the channels are not open in the intact cell. But why not? At least a part of the answer may lie in the different SUR isoform found in the heart – SUR2A subunits may fail to activate cardiac channels. There are additional confusing consequences of transgene expression. Heart rate is reduced in Kir6.2[DN,K185Q] transgenic mice, but contractility is elevated in isolated hearts. Why is this? A slight AP prolongation during mid-repolarization correlates with enhanced L-type Ca^{2+} current in these myocytes. Is enhanced Ca^{2+} current driving enhanced contractility in the face of reduced heart rate? Or is enhanced contractility, secondary to enhanced Ca^{2+} current, driving down the heart rate?

In summary, both knock-out of K_{ATP} channels, and transgenic expression of mutant channels, has generated dramatic phenotypic changes in cardiac and β-cells. As will be discussed, the results have confirmed or enhanced some paradigms, but there are some surprising and as yet unexplained phenomena to be considered.

From TRPV4 to TRPV6: mechanisms of activation and inactivation

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Cation channels of the trp-superfamily provide a ubiquitously used pathway for Ca^{2+} entry. This family can be subdivided into the receptor-operated short (canonical) TRPC channels, the long TRPM (melastatin) channels, and a subfamily, TRPV, which is related to the vanniloid receptor VR-1. Since cloning of the capsaicin receptor VR-1 (TRPV1), five related proteins have been identified which show a surprising diversity in their mechanisms of activation. Focus of this communication is on TRPV4 (OTRPC4, VR-OAC, TRP12) and the highly Ca^{2+}-selective channels TRPV5 and -6 (ECaC1, ECaC2 or CaT1).

TRPV4, heterologously expressed in HEK 293 cells, is a Ca^{2+}- and Mg^{2+}-permeable cation channel, which only weakly discriminates between monovalent and divalent cations (P_{Ca}/P_{Na}~6.9, P_{Mg}/P_{Na}~2.5). TRPV4 is activated by cell swelling but much more efficient by a ligand, 4α-PDD (phorbol 12,13-didecanoate). Candidates for endogenous ligands refer to anandamide related compounds. Increasing the temperature above 30 °C activates TRPV4. Both types of activation require an intact N-terminus which comprises at least three ankyrin binding repeats. Deletion of one single domain already reduced the ligand activation but not activation by cell swelling of TRPV4. Activation by the ligands and by heat shows desensitisation during repetitive activation. Interestingly, activation is modulated by exchanging single amino acids in the pore region. Activation of TRPV4 is also modulated by a potential-dependent open pore block by extracellular Ca^{2+}, which is bound within the pore at an identified low affinity binding site. As a probe for dissecting TRPV4 activation from co-activation of other ion channels, distinct properties of a submicromolar voltage-

dependent block by ruthenium red will by described. TRPV4 is inactivated by an increase of the intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$. IC_{50} for this inactivation is approximately 450 nM at −80 mV and is dramatically changed by mutations in the sixth transmembrane loop (TM6). This Ca^{2+}-dependent inactivation is much less efficient than for the highly Ca^{2+}-selective channels TRPV5 and -6.

All features of activation can be measured in freshly isolated mouse aorta endothelial cells, which endogenously express TRPV4. Activation of TRPV4 in those cells is accompanied by an increase in $[Ca^{2+}]_i$, indicating that these channels are involved in endothelial Ca^{2+} signalling triggered by diverse physical and chemical stimuli. Possible functional consequences will be discussed.

Gating of TRPV5 and -6, which are both highly Ca^{2+} selective channels, involve as a critical step the removal of blocking Mg^{2+} or Ca^{2+} ions from a high-affinity binding site within the pore. This step requires hyperpolarisation of the membrane. Both TRPV channels sensitively inactivate by an increase in $[Ca^{2+}]_i$. This Ca^{2+}-dependent inactivation occurs with an IC_{50} of approximately 120 nM. Three regions in the C terminus have been identified which are necessary for Ca^{2+} dependent inactivation of TRPV5 and -6. The very slow recovery from inactivation hints to the possible interaction with a modulating protein. In addition, it will be shown that the inactivation includes as a critical determinant the intracellular linker between TM2 and TM3. This linker seems also to determine the differences in the inactivation behaviour between TRPV5 and -6 and is responsible for distinct phenotypes of the both channels types in relation to the speed of the fast initial inactivation at hyperpolarizing steps, the degree of inactivation and the Ca^{2+} over Ba^{2+} discrimination. So far, no ligand activation for TRPV5 and -6 could be identified. A model for gating of TRPV5/6 will be discussed.

Structure and function of TRP-related gene products

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The TRP superfamily includes > 20 related cation channels that play critical roles in processes ranging from sensory physiology to vasorelaxation and altered sexual and social behaviours. Defects in TRP channels have been associated with changes in growth control, and other TRP-related proteins appear to be prognostic markers for metastasis in patients with localized malignant melanoma and the progression in prostatic cancer. TRP proteins are widely expressed in the nervous system, and, in non-excitable cells, TRP-related channels may be the primary mode of Ca entry. TRP proteins are cation channels; however, they vary significantly in their selectivity and mode of activation. In addition, at least one of these channel proteins, PKD2 or TRPP2, appears to be a endoplasmic reticulum Ca release channel. Nevertheless, members of the TRP superfamily share significant sequence homology and predicted structural similarities, such as six predicted transmembrane segments.

The current focus of our work is on TRPC4, a member of the TRPC subfamily, and on TRPV6 (formerly called CaT-like), a member of the TRPV subfamily. TRPC4 has been implicated in store-operated Ca-entry in various cells and endothelial cells – both macrovascular and microvascular – of mice deficient in TRPC4 lack a store-operated Ca current. As a consequence, agonist-induced Ca entry and vasorelaxation is reduced markedly, showing that TRPC4 is an indispensable component of store-operated channels in native endothelial cells and that these channels directly provide a Ca-entry pathway essentially contributing to blood vessel tone and microvascular endothelial permeability. The TRPC4 protein contains three binding sites for Ca-calmodulin and is part of a signalling complex, which also contains phospholipase C and is assembled by the divalent PDZ-domain protein NHERF (synonym ezrin binding protein 50).

TRPV6 is abundantly expressed in acinar cells of the pancreas, myoepithelial cells of the salivary gland and trophoblasts and syncytiotrophoblasts of the placenta. It encodes a cation channel which is highly Ca selective. TRPV6 is also expressed in locally advanced prostate cancer, metastatic and androgen-insensitive prostatic lesions, but is undetectable in healthy prostate tissue and benign prostatic hyperplasia. Accordingly, a molecular classification of prostate cancer subclasses and class prediction by monitoring the level of human TRPV6 gene expression may be feasable. In addition, the TRPV6 channel may serve as a target for therapeutic strategies in cancer treatment.

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