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Review

Intracellular potassium and chloride channels: An update[★✪]

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Channels selective for potassium or chloride ions are present in all intracellular membranes such as mitochondrial membranes, sarcoplasmic/endoplasmic reticulum, nuclear membrane and chromaffin granule membranes. They probably play an important role in events such as acidification of intracellular compartments and regulation of organelle volume. Additionally, intracellular ion channels are targets for pharmacologically active compounds, e.g. mitochondrial potassium channels interact with potassium channel openers such as diazoxide. This review describes current observations concerning the properties and functional roles of intracellular potassium and chloride channels.

Ion channels selective for potassium or chloride ions exist in all intracellular membranes. They have been described in mitochondrial membranes, endoplasmic/sarcoplasmic reticulum, nuclear membrane and chromaffin granule membranes (for review see Szewczyk, 1998). After a long period of identification of different ion channels we are now beginning to understand their functional roles within the cells. For example, mitochondrial ion channels seem to play an important role in such cellular events as exocytosis (Giovannuci *et al.*, 1999) and synaptic transmission (Jonas *et al.*, 1999). Recently, potassium channel openers (KCOs) acting on mitochondria attracted attention due to their possible role in ischemic preconditioning in the heart (O'Rourke, 2000). Additionally, Dent's disease is caused by a mutation in the renal chloride channel gene,

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Abbreviations: mitoK_{ATP} channel, mitochondrial ATP-regulated potassium channel; K_{ATP} channel, plasma membrane ATP-regulated potassium channel; 5-HD, 5-hydroxydecanoic acid; PKC, protein kinase C; KCOs, potassium channel openers; CLIC, intracellular chloride channel; TNFα, tumor necrosis factor α.

clcn5, which encodes a channel protein present in intracellular vesicles such as endosomes (Gunther *et al.*, 1998).

MITOCHONDRIAL POTASSIUM AND CHLORIDE CHANNELS

In 1991 the mitochondrial ATP-regulated potassium channel (mitoK_{ATP} channel), sensitive to glibenclamide, was identified in the inner membrane of rat liver mitochondria (Inoue *et al.*, 1991). Its activity was later identified in the inner membrane of beef heart mitochondria (Paucek *et al.*, 1992). The molecular identity of mitoK_{ATP} channels is unknown (for a review see Szewczyk & Marban, 1999). Several observations on the pharmacological profile and immunofluorescence may suggest that the mitoK_{ATP} channel is similar to the plasma membrane ATP-regulated potassium channel (K_{ATP} channel) and belongs to the inward rectifier K^+ channel family such as Kir6.x (Suzuki *et al.*, 1997). Recently, Kir6.1 was also localised in rat brain mitochondria (Zhou *et al.*, 1999). Properties of the mitoK_{ATP} channel have recently been reviewed in detail (Bernardi, 1999; Szewczyk & Marban, 1999; Kicińska *et al.*, 2000). In this review we focus on the most recent observations concerning this protein.

A variety of reports have been published suggesting that the mitoK_{ATP} channel plays an important role in cardioprotection (Gross & Fryer, 1999; Garlid, 2000; Sato & Marban, 2000; Gross, 2000; Downey & Cohen, 2000). For example, the potassium channel opener nicorandil, a potent cardioprotective agent, was also shown to act by opening the mitoK_{ATP} channel in cardiac myocytes (Sato *et al.*, 2000). Pretreatment with an inhibitor of the mitoK_{ATP} channel, 5-hydroxydecanoic acid (5-HD), before ischemic preconditioning partially abolished cardioprotection. Moreover, it has been shown that rats subjected to ischemia–reperfusion synthesise much less ATP than control animals and ischemic preconditioning significantly increases ATP synthesis (Fryer *et al.*, 2000). These data are consistent with

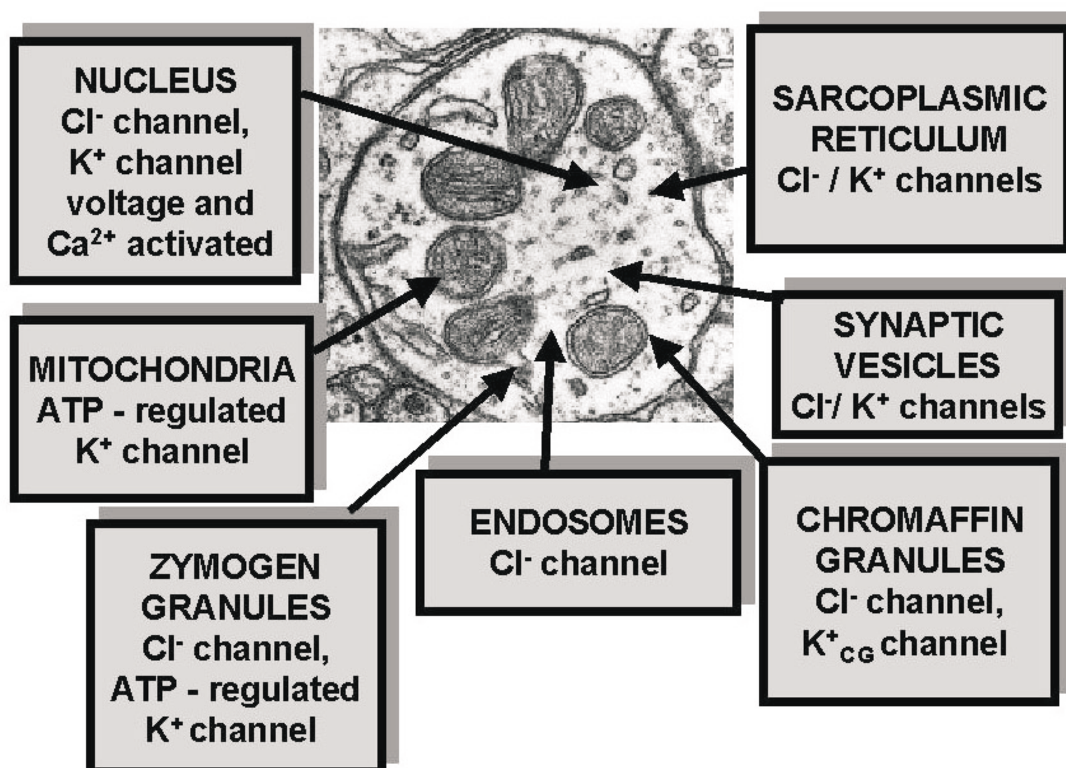

Figure 1. Distribution of intracellular potassium and chloride ion channels within the cell.

the notion that inhibition of mitoK_{ATP} channels abolished the effect of ischemic preconditioning induced protection of mitochondrial function. Recently, evidence for the mitoK_{ATP} channels as effectors of human myocardial preconditioning was obtained (Ghosh *et al.,* 2000; Carroll & Yellon, 2000).

Preservation of mitochondrial function by diazoxide during sustained ischemia in rat heart has been observed (Iwai *et al.,* 2000). It was shown that hypoxia induces a decrease in the mitochondrial oxygen consumption rate of myocardial skinned bundles to approximately 40% of the pre-hypoxic value. In contrast, treatment of the bundles with diazoxide preserves the normal mitochondrial oxygen consumption rate during hypoxia. This effect was abolished by a combined treatment with either glibenclamide or 5-HD (Iwai *et al.,* 2000).

Activation of the mitoK_{ATP} channel by nitric oxide was shown (Sasaki *et al.,* 2000). The NO donor *S*-nitroso-*N*-acetyl-DL-penicillamine (SNAP) oxidised the mitochondrial matrix dose-dependently without activating the plasma membrane K_{ATP} channel. SNAP-induced oxidation was blocked by 5-HD and by an NO scavenger. The conclusion of this study was that NO directly activates mitoK_{ATP} channels and potentiates the ability of diazoxide to open these channels. Recently, the effects of KCOs on intracellular Ca^{2+} concentration and mitochondrial potential in cultured rat hippocampal neurons have been studied (Jakob *et al.,* 2000). It was shown that pretreatment with the KCOs cromakalim and diazoxide increased the expression level of proteins involved in apoptosis. These results also suggest that mitoK_{ATP} channels are present in hippocampal neurons and may confer neuroprotection by altering Bcl-2 and Bcl-X_L expression levels (Jakob *et al.,* 2000). Recently, depolarization of hippocampal mitochondria by the KCOs diazoxide and RP66471 was shown (Dębska *et al.,* 2001).

Additionally, large conductance of the BK-type potassium channel was observed by patch-clamp techniques in the inner mitochondrial membrane of the human glioma cell line LN229 (Siemen *et al.,* 1999). This channel, with the conductance of 295 pS, shows similarities to the BK-type $K_{(Ca)}$ channel of the plasma membrane found in several tissues, i.e. it is activated by Ca^{2+} and blocked by charybdotoxin (Siemen *et al.,* 1999).

Recently, a novel intracellular chloride channel (mitoCLIC) has been identified in mitochondria (Fernández-Salas *et al.,* 1999). The mitoCLIC cDNA is very similar to several reported sequences in the GeneBank^{TM}/EMBL/Protein Data Bank database. It is similar to several plasma membrane chloride channels and intracellular chloride channels (Fernández-Salas *et al.,* 1999). The mitoCLIC cDNA codes for a 253 amino acid protein with a predicted molecular mass of 27.8 kDa (Fernández-Salas *et al.,* 1999). The mitoCLIC protein also shows extensive homology with a family of chloride channels, especially the intracellular p64H1 chloride channel present in the endoplasmic reticulum of rat brain (98% homology) (Duncan *et al.,* 1997) and human p64H1 (Chunag *et al.,* 1999). An analysis of the mitoCLIC protein revealed two putative transmembrane domains and possible cAMP-dependent protein kinase phosphorylation sites, several PKC, CK2 and tyrosine kinase phosphorylation sites, and N-myristoylation sites (Fernández-Salas *et al.,* 1999).

The mitoCLIC mRNA is expressed to the greatest extent *in vivo* in the heart, lung, liver, brain and skin. However, it was detected in all tissues tested (Fernández-Salas *et al.,* 1999). This protein is the first mitochondrial ion channel shown to be differentially regulated. The expression in the heart, lung, liver, kidney and skin is higher in $p53^{+/+}$ mice than in $p53^{-/-}$ mice by 2.5- to 5-fold, but these differences were not detected in the intestine, spleen, testis and kidney (Fernández-Salas *et al.,* 1999). Also in keratinocytes in culture the levels of the mitoCLIC mRNA and protein are higher in $p53^{+/+}$ than in $p53^{-/-}$ cells and increase further after the induction of differentiation of keratinocytes (Fernández-Salas *et al.,* 1999). Moreover, overexpression of p53 in primary mouse keratinocytes increases the mitoCLIC mRNA and protein level. Exogenous human TNFα also increases the levels of the mitoCLIC mRNA and protein in both $p53^{+/+}$ and

$p53^{-/-}$ keratinocytes (Fernández-Salas *et al.,* 1999).

CLIC-5 CHLORIDE CHANNEL

The intracellular chloride channels (CLICs) have been implicated in chloride ion transport within various subcellular compartments. Recently, molecular, and biochemical characterization of a new member of this family termed CLIC-5 were reported (Berryman & Bretscher, 2000). CLIC-5 was isolated from extracts of placental microvilli as a component of a multimeric complex consisting of several known cytoskeletal proteins, including actin and gelsolin. Human cDNAs were cloned and antibodies specific for CLIC-5, CLIC-1/NCC27, and CLIC-4/huH1/p64H1 were generated. CLIC-5 shares 52–76% overall identity with human CLIC-1, CLIC-2, CLIC-3, and CLIC-4. Northern blot analysis showed that CLIC-5 has a distinct pattern of expression compared with CLIC-1 and CLIC-4. Immunoblot analysis of extracts from placental tissues demonstrated that CLIC-4 and CLIC-5 are enriched in isolated placental microvilli, whereas CLIC-1 is not. Moreover, in contrast to CLIC-1 and CLIC-4, CLIC-5 is associated with the detergent-insoluble cytoskeletal fraction of microvilli. Indirect immunofluorescence microscopy revealed that CLIC-4 and CLIC-5 are concentrated within the apical region of the trophoblast, whereas CLIC-1 is distributed throughout the cytoplasm. These studies suggest that CLIC-1, CLIC-4, and CLIC-5 play distinct roles in chloride transport and that CLIC-5 interacts with the cortical actin cytoskeleton in polarized epithelial cells.

CHLORIDE CHANNEL AND DENT'S DISEASE

Dent's disease is an X-linked renal tubular disorder characterised by low molecular mass proteinuria, hypercalciuria, nephrocalcinosis, nephrolithiasis, and renal failure. The disease is caused by mutations in a renal chloride channel gene, *clcn5,* which encodes a 746 amino acid protein CLC-5 with 12 to 13 transmembrane domains, present in renal proximal tubule endosomes. Recently, six unrelated patients with Dent's disease were identified and investigated for *clcn5* mutations by DNA sequence analysis of the 11 coding exons of *clcn5* (Yamamoto *et al.,* 2000). This investigation revealed mutations such as a nonsense mutation (Tyr617Stop) and an A to T transversion at codon 601 that would result in either a missense mutation (Asp601Val) or creation of a novel donor splice site. In addition, these mutations were confirmed by restriction endonuclease digestion or sequence-specific oligonucleotide hybridization and were not common polymorphisms. These mutations are likely to result in truncated CLC-5 and a loss of its function, and these findings expand the spectrum of *clcn5* mutations associated with Dent's disease.

Recently, the subcellular localization and function of CLC-5 were investigated in the LLC-PK1 porcine proximal tubule cell line (Dowland *et al.,* 2000). A cDNA for the porcine CLC-5 ortholog (pCLC-5) predicted to encode an 83 kDa protein with 97% amino-acid sequence identity to rat and human CLC-5 has been cloned. By immunofluorescence, pCLC-5 was localized in early endosomes of the apical membrane fluid-phase endocytotic pathway and in the Golgi. Xenopus oocytes injected with pCLC-5 cRNA exhibited outwardly rectifying whole cell current. Acidification of the extracellular medium reversibly inhibited this outward current. Overexpression of CLC-5 in LLC-PK1 cells resulted in morphological changes, including loss of cell–cell contacts and the appearance of multiple prominent vesicles. These findings are consistent with a potential role for CLC-5 in the acidification of membrane compartments of both the endocytic and the exocytic pathway, and suggest that its function may be important for normal vesicular trafficking.

INTRACELLULAR CHLORIDE CHANNEL IN THE HIPPOCAMPUS

A novel class of intracellular chloride channels, the p64 family, has been found in several types of intracellular membranes (Duncan *et al.,* 1997).

These channels, probably acting in concert with the electrogenic proton pump, regulate the pH of the vesicle interior, which is critical for vesicular function. Recently, molecular cloning of p64H1, a p64 homolog, from both human and bovine has been described (Chuang *et al.,* 1999). Northern blot analysis showed that p64H1 is expressed abundantly in the brain and retina, whereas the other members of this family (e.g., p64 and NCC27) are expressed only at low levels in these tissues. Immunohistochemical analysis of p64H1 in rat brain, using an affinity-purified antibody, revealed a high level of expression in the limbic system of hippocampal formation, amygdala, hypothalamus and septum. Immunoelectron microscopic analysis of p64H1 in hippocampal neurons demonstrated a striking association between p64H1 and large dense-core vesicles (LDCVs) and microtubules. In contrast, very low p64H1 labeling was found associated with small synaptic vesicles (SSVs) in axonal profiles. Immunoblot analysis confirmed that p64H1 is colocalized with heavy membrane fractions containing LDCVs rather than the fractions containing SSVs. These results suggest that p64H1-mediated Cl^- permeability may be involved in the maintenance of low internal pH in LDCVs and in the maturation of LDCVs and the biogenesis of functional neuropeptides (Chuang *et al.,* 1999).

PARCHORIN: A NOVEL INTRACELLULAR CHLORIDE CHANNEL?

A 120 kDa phosphoprotein has previously been reported to translocate from cytosol to the apical membrane of gastric parietal cells in association with stimulation of HCl secretion (Urushidani *et al.,* 1999). To determine the molecular identity of the protein, its expression pattern in different tissues was studied followed by cloning of the corresponding cDNA (Nishizawa *et al.,* 2000). Immunoblot analysis showed that the 120 kDa phosphoprotein was highly enriched in tissues that secrete water, such as parietal cell, choroid plexus, salivary duct, lacrimal gland, kidney, and airway epithelia. This protein was named „parchorin“ based on its highest enrichment in parietal cells and choroid plexus. cDNA for parchorin from rabbit choroid plexus coding for a protein consisting of 637 amino acids with a predicted molecular mass of 65 kDa was obtained. The discrepancy in size on SDS/PAGE was considered to be due to its highly acidic nature. Parchorin is a novel protein that has significant homology to the family of intracellular chloride channels, especially to the chloride p64 channel from bovine kidney. When expressed as a fusion protein with green fluorescent protein (GFP) in the LLC-PK1 kidney cell line, GFP-parchorin, unlike other CLIC family members, existed mainly in the cytosol. Furthermore, when Cl^- efflux from the cell was elicited, GFP-parchorin translocated to the plasma membrane. These results suggest that parchorin plays a critical role in water-secreting cells, possibly through the regulation of chloride ion transport (Urushidani *et al.,* 2000).

NUCLEAR MEMBRANE ION CHANNELS

Extracellular signal-regulated kinase 7 (ERK7) is a member of the mitogen-activated protein kinase family. It has a carboxyl-terminal tail that is required for proper cellular localization and protein ability to inhibit DNA synthesis (Abe *et al.,* 1999). To identify proteins that interact with ERK7, a yeast two-hybrid screen with the carboxyl-terminal tail of ERK7 as bait was used (Qian *et al.,* 1999). As a result cDNA for a novel protein termed CLIC-3 was isolated. The interaction between CLIC-3 and ERK7 in mammalian cells was confirmed by co-immunoprecipitation. CLIC-3 has significant homology to human intracellular chloride channels 1 (NCC27/CLIC-1) and 2 and bovine kidney chloride channel p64. Like NCC27/CLIC-1, CLIC-3 is predominantly localized to the nucleus and stimulates chloride conductance when expressed in cells. Taken together, these results suggest that CLIC-3 is a new member of the human CLIC family. The interaction observed between CLIC-3 and ERK7 is the first demonstration of a stable complex between a chloride ion channel and a member of the mitogen-activated protein kinase family. The specific association of CLIC-3 with the carboxyl-terminal tail of ERK7

suggests that CLIC-3 may play a role in the regulation of cell growth (Qian *et al.,* 1999).

Recently, using patch-clamp techniques on isolated nuclei, the ion channels in the outer nuclear envelope of T-cell (human Jurkat) and BFL5 cell (murine promyelocyte) lines were measured (Franco-Obregon *et al.,* 2000). In the patch-clamp recordings of Jurkat nuclear membranes, Cl^- channels showed the conductance of 105 pS. Nucleotides transiently inhibited these anion channels. In contrast, BFL5 nuclear channels were cation selective with the conductance of 52 pS and were insensitive to GTP. It was hypothesized that the T- and B-cell nuclear membrane channels are distinct, which might be related to their unique roles in the immune system (Franco- Obregon *et al.,* 2000).

CHLORIDE CHANNEL AS TARGET FOR TYROSINE KINASE

P64 is a chloride channel of intracellular membranes which is present in secretory vesicles (Al-Awqati *et al.,* 1992). Mechanisms by which the p64 channel could be regulated are largely unknown. p59fyn is a non-receptor tyrosine kinase of the src family that has been implicated in a variety of intracellular signalling events. The N-terminal portion of p64 has several potential binding sites for SH2 domains of src family. It was demonstrated that p64 becomes tyrosine phosphorylated when co-expressed with p59fyn in HeLa cells (Edwards & Kapadia, 2000). Using site-directed mutagenesis, it was found that p64 tyrosine 33 was necessary for SH2 binding. It was also observed that a small fraction of native kidney p64 could bind fyn SH2 *in vitro*. Immunoprecipitation of p64 from solubilized kidney membranes yielded a kinase activity with the same mobility on SDS/PAGE as authentic bovine p59fyn. Finally, it was demonstrated that co-expression of p64 and p59fyn in HeLa cells resulted in enhanced p64-associated chloride channel activity (Edwards & Kapadia, 2000).

FINAL REMARKS

Over last year genes of a number of intracellular ion channels have been identified, thus ending a long period limited to phenomenology in this field. This open a new possibilities to study the structure and function of intracellular ion channels. Observations concerning the role of mitoK_{ATP} channels in ischemic preconditioning also open new perspectives for this field. For example, ischemic preconditioning induced by mitochondrial KCOs has recently emerged as a new strategy for improving the preservation of globally ischemic cold-stored hearts during cardiac transplantation (Ahmet *et al.,* 2000; Kevelaitis *et al.,* 1999). All these suggest that intracellular ion channels may be important targets for pharmacological treatments. Hence, the field of intracellular ion channels will probably attract more attention in the coming years.

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