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Properties and regulation of ion channels in MDCK cells

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Madin-Darby canine kidney (MDCK) cells are a permanent cell line derived from dog kidney with properties similar to distal nephron [1–5]. The cells are widely used as a model cell line for studies on epithelial polarization [6–9], formation and regulation of tight junctions [10–12] epithelial transport [13–15], mechanisms of infection [16–18], etc. Furthermore, the cells have proven useful in the study of the properties and regulation of ion channels. This review will focus on the latter aspect of MDCK cells. After a brief synopsis of the transport systems encountered in MDCK cells and of the basic electrical properties, the functional characteristics and regulation of ion channels in MDCK cells will be described.

Transport systems and electrical properties of MDCK cells

Transport systems

If grown to confluency on impermeable culture dishes, MDCK cells display net ion and fluid transport from the apical to the basolateral cell side, leading to the formation of domes, fluid filled blisters between epithelial cell layer and culture dish [19–21]. On the other hand, MDCK cells are capable of secreting H^+ [22] and Cl^- [23]. The following cellular transport systems are involved: at the apical cell membrane, a Na^+/H^+ exchanger [24–29] operates in parallel to a H^+/K^+ ATPase [30], a Cl^-/HCO_3^- exchanger [26, 31], Cl^- and K^+ channels.

At the basolateral cell membrane, a $Na^+,K^+,2Cl^-$ cotransport [4, 32, 33] operates in parallel to the Na^+/K^+ ATPase [34–36], a Na^+/Ca^{2+} exchanger [37, 38], a Na^+/H^+ exchanger [39–41], K^+ and probably Ca^{2+} channels.

Similar transport processes are expressed in subconfluent MDCK cells [42].

Further transport systems identified in confluent MDCK cells include amino acid transporters systems A, ASC and L [43], Na^+ and Cl^- dependent taurine transporter [44], a lactate, H^+ cotransporter [45], and a xenobiotic secretory transport system [46].

Electrical properties

The potential difference across the cell membrane (PD) of MDCK cells is some −50 mV in both confluent [47, 48] and subconfluent [49] MDCK cells. The input resistance, which comprises cell membrane resistance and gap junctional resistance, is again similar (40 to 60 MΩ) in confluent [47, 48] and subconfluent [50] MDCK cells. Cable analysis in subconfluent MDCK cells [49] allowed the calculation of the cell membrane resistance (2.0 ± 0.2 $k\Omega cm^2$) and of the intercellular resistance (6.1 ± 0.8 MΩ). The cell membrane resistance displays slight rectification because it decreases upon depolarizing currents [49]. The cell membrane is mainly conductive to K^+ [50] and to Cl^- [51]. Bicarbonate influences the cell membrane potential mainly by modifying K^+ conductance; a step decrease of bicarbonate depolarizes the cell membrane presumably by cellular acidification [50].

The confluent MDCK cell layers form tight junctions, which are preferably permeable to Na^+. The transepithelial resistance may vary from some 200 Ωcm^2 to 4000 Ωcm^2, depending on the cell type studied [20, 47, 52, 53].

Properties of single ion channels in MDCK cells

K^+ channels

Three intermediate conductance K^+ channels (31 pS, 89 pS, 109 pS) and a maxi-K^+ channel (220 pS) have been identified in the apical cell membrane of confluent MDCK cells [54]. A further intermediate conductance K^+ channel (26 pS) has been found in the basolateral cell membrane. In subconfluent cells, a maxi-K^+ channel (260 pS) and much more frequently an intermediate conductance (60 pS) K^+ channel have been described [42, 55–57]. The latter two channels are sensitive to intracellular Ca^{2+} and are thus activated by a number of hormones, which increase intracellular Ca^{2+} activity [42, 55, 56]. The activation of the K^+ channels does not require the participation of calmodulin [58]. Instead, the calmodulin antagonist itself leads to activation of the Ca^{2+}-sensitive K^+ channels and hyperpolarization of the cell membrane, most likely due to its enhancing effect on intracellular Ca^{2+} activity [58].

Anion channels

At the apical cell membrane of confluent MDCK cells, an anion channel of 460 pS has been identified, which is activated upon sudden changes of the potential difference across the cell membrane patch [59]. At the basolateral cell membrane, on the other hand, a 46 pS Cl^- channel has been described [60]. In subconfluent MDCK cells, no spontaneous Cl^- channel activity could be detected with single channel recording, even though conventional electrophysiology points to the existance of Cl^- conductance in the cells [51]. However, an unselective anion channel of 30 to 60 pS appears following swelling of the cells, as detailed below.

Other channels

In confluent MDCK cells Na^+ and Ca^{2+} currents have been found negligible [55]. Similarly, no evidence for Na^+ or Ca^{2+} conductance has been found in subconfluent MDCK cells [39]. However, the step reduction of extracellular Na^+ lead to a hyperpolarization of the cell membrane by reversal of the $Na^+/$

Ca^{2+} exchange, increase of intracellular Ca^{2+} activity and subsequent activation of Ca^{2+}-sensitive K^+ channels [38]. Nevertheless, several mediators, such as epinephrine [61], nucleotides [38, 62] or acetylcholine [63] as well as *E. coli* heat stable enterotoxin B [64] stimulate the rapid entry of Ca^{2+} into the cells, presumably via receptor operated Ca^{2+} channels.

Structure, function and regulation of I_{Cln}

Structure and function of I_{Cln}

Using mRNA extracted from MDCK cells a protein was cloned that, when expressed in *Xenopus laevis* oocytes, leads to an outwardly rectifying chloride current [65]. The cDNA coding for the protein (I_{Cln}) is 1579 bp long comprising a 71 bp 5′ untranslated region, an 803 bp 3′ untranslated region and open reading frame (ORF) of 705 bp coding for a 235 amino acid (AA) long protein with an apparent molecular size of $\approx$ 40 kD in a denaturing SDS gel. After injection of the mRNA coding for I_{cln} into oocytes and after a time period of approximately one to two days, a current can be measured that reverses at ≈ -30 mV and inactivates at potentials more positive than +40 mV. Using substances known to impede chloride currents, such as DIDS or NPPB, an inhibition of the expressed current can be measured. However, nucleotides added to the extracellular fluid can, in addition, block the current in a dose-dependent manner within seconds, with cGMP, cAMP, ATP and ITP being most effective [65]. A similar nucleotide sensitivity of chloride channels was shown in tracheal, colonic and heart cells [66–68]. The AA sequence and the deduced model of the I_{Cln} channel protein at the predicted extracellular site revealed a glycine-repeat which resembles a 'walker' motif for nucleotide binding [65]. Mutations of this putative nucleotide binding region lead to a fully expressed current, but with a marked reduction of nucleotide sensitivity (1 mM cAMP which led to a reduction of the wild type I_{Cln} current to $\approx$ 30% had no effect in a mutation in which all three glycines were mutated into alanines). Furthermore, the kinetics of the mutated but fully expressed current was changed. The current no longer showed inactivation at potentials higher than +40 mV but, in contrast, impresses with a further activation with a τ of $\approx$ 100 ms at +40 mV.

Putting these data together the above-mentioned experiments can be simply explained by I_{Cln} being the channel itself. However, we cannot rule out I_{Cln} being a regulator of an inherent channel protein. Interestingly, the I_{Cln} protein is found to be more abundant in the cytosol rather than the membrane fraction of MDCK cells and NIH 3T3 fibroblasts (U.O. Nagl and M. Paulmichl, unpublished results). Similar results were obtained using *Xenopus laevis* oocytes [69] and were taken as evidence that I_{Cln} might be a regulating protein. However, it is known from other water soluble proteins that they can be introduced into the plasma membrane and form channels [70, 71] under native conditions.

Regulation of I_{Cln}

The kinetics of the current (inactivation at potentials more positive than +40 mV) resembles the chloride currents activated after cell swelling. In addition to the kinetics the selectivity of the I_{Cln} current also corresponds to the swelling-induced chloride current, with SCN^- being conducted best of the tested ions, followed by I^-, Br^- and Cl^-. The expressed channels are virtually impermeable for gluconate [72]. The expressed I_{Cln} current in oocytes is sensitive to changes of the proton concentration [73]. Reduction of the pH significantly increases the newly expressed chloride current. This pH sensitivity of the I_{Cln} current could be one of the mechanisms by which this protein becomes activated after cell swelling. It has been shown that reduction of the osmolarity leads to a significant acidification of the cytosol due to an extrusion of HCO_3^-.

We investigated whether I_{Cln} is involved in the swelling-induced activation of chloride channels. Because all the cells tested expressed I_{Cln} at the protein level and showed a swelling-induced chloride current an overexpression of I_{Cln} on top of the endogenous one did not seem to give a straightforward answer on how and if at all I_{Cln} is involved in the swelling-induced activation of chloride currents. Using antisense oligonucleotides against the first nucleotides at the beginning of the ORF coding for I_{Cln}, we showed that I_{Cln} is of paramount importance for the activation of chloride currents after cell swelling [74, 75]. Using antibodies against a fusion protein of I_{Cln} a similar conclusion was drawn [69]. Under the influence of antisense oligonucleotides the I_{Cln} protein seems to vanish, the swelling-induced current is dramatically reduced, and the chloride flux measured with a chloride-sensitive dye after cell swelling [76] is decreased. As previously shown, reduction of the osmolarity by 50 mOsm (omitting mannitol) leads in MDCK cells to the activation of a chloride current indistinguishable from that activated in NIH 3T3 fibroblasts [76]. MDCK cells are able to effectively regulate their cell volume after swelling. From the experiments mentioned above it can be deduced that I_{Cln} is the major protein involved in the swelling-induced activation of chloride channels. It is believed that ion channels are essential in the first step of volume regulation, and that changes in the osmolyte concentration are responsible for the long lasting regulatory effect. I_{Cln} could gain some interest if a link to the volume-sensitive anion channels which are described to mediate the efflux of inositol and taurine in swollen cells [77] were established.

Regulation of ion channels in MDCK cells

Hormones

Transcellular transport in confluent MDCK cells is stimulated by aldosterone [26, 30, 78, 79], antidiuretic hormone [4, 80], prostaglandin E [4, 19, 52, 81], epinephrine [23, 80, 82], acetylcholine [83], kinins [84–86], ATP 24,83] and glucagon [4]. From these hormones prostaglandin E, acetylcholine, epinephrine, bradykinin and ATP lead to marked electrophysiological effects in subconfluent MDCK cells; all mediators markedly hyperpolarize the cell membrane. The hyperpolarizing effect of epinephrine [61, 87], acetylcholine [88] and ATP [62, 63] is sustained, and the hyperpolarizing effect of prostaglandin E [89] and bradykinin [90–92] is only transient. In addition, serotonin [93] hyperpolarizes the cell membrane transiently. A transient hyperpolarization does not necessarily reflect a transient effect of the mediator, as shown for bradykinin, which produces a sustained decrease of cell membrane resistance [92]. Thus, bradykinin leads to a transient activation of K^+ channels and to sustained activation of another conductance, presumably an anion conductance.

The hyperpolarizing mediators enhance intracellular Ca^{2+} activity and activate Ca^{2+}-sensitive K^+ channels [55–58, 91, 94–96]. Thus, at first glance, all mediators appear to exert their hyperpolarizing effects via a uniform cellular mechanisms, that is, activation of phospholipase C, formation of inositoltrisphosphate

($InsP_3$) and inositoltetrakisphosphate ($InsP_4$), stimulation of cellular Ca^{2+} relase and/or entry of Ca^{2+} from the extracelluar space. However, a closer analysis of the cellular mechanisms involved revealed distinct differences between the hormones and pointed to additional mechanisms involved in K^+ channel activation by these hormones. The apparent gain of Ca^{2+} increase per $InsP_3$ formed was different between the three hormones epinephrine, ATP and bradykinin [97]. Furthermore, the hyperpolarizing effects of serotonin and acetylcholine were abolished by pretreatment of the cells with pertussis toxin, while the hyperpolarizing effect of epinephrine was rendered transient and the effect of ATP and bradykinin seemingly unaffected by the same treatment [98], even though pertussis toxin markedly inhibited the stimulation of $InsP_3$ formation by the last three hormones [58, 87, 91]. It must be kept in mind that the electrophysiological experiments, the Ca^{2+} measurements and the determinations of $InsP_3$ could not be done under strictly identical conditions and the comparison may suffer from any difference in treatment. Nevertheless, the data strongly suggest that additional mechanisms are involved in channel activation by these hormones.

In contrast to the aforementioned hormones, progesterone leads to a sustained depolarization of the cell membrane, paralleled by a decrease of K^+ selectivity and an increase of cell membrane resistance [99]. Thus, progesterone depolarizes the membrane of MDCK cells by inhibition of K^+ channels.

Very little is known about hormonal regulation of anion channels in MDCK cells. The stimulation of Cl^- secretion is thought to involve activation of Cl^- channels, which are presumably activated by cAMP. In fact cAMP does depolarize the cell membrane and increase its Cl^- selectivity [51], and cAMP shrinks MDCK cells presumably by stimulating loss of KCl [100]. The channels involved have, however, not yet been characterized by single channel analysis.

Cell volume

Upon osmotic cell swelling, subconfluent MDCK cells respond with a variable transient hyperpolarization followed by a sustained depolarization of the cell membrane [101, 102]. The hyperpolarization is due to transient activation of the Ca^{2+}-sensitive inwardly rectifying K^+ channel of some 60 pS, the depolarization due to the activation of an unselective anion channel of some 30 to 60 pS [103, 104]. The K^+ channel activation is probably secondary to enhanced cellular Ca^{2+} activity [105]. The anion channel, which in confluent cells probably sits in the basolateral cell membrane is more conductive to HCO_3^- than to Cl^- (3:1) [103], and the cells indeed lose HCO_3^- during cell swelling [106]. Moreover, the channel may allow the passage of other anions such as phosphate, or even amino acids such as aspartate, glutamate and taurine [104, 107], which are released upon cell swelling in parallel to KCl [108–110].

Osmotic shrinkage of MDCK cells leads to an increase of cell membrane resistance [111], probably reflecting the inhibition of both anion channels and K^+ channels. The increase in cell membrane resistance is paralleled by a mild hyperpolarization of the cell membrane, indicating that the K^+ channels are less affected than depolarizing channels such as anion channels [111, 112]. The inhibition of the channels impairs transepithelial transport [113]. The cells accumulate ions by activation of Na^+,K^+,$2Cl^-$ cotransport, Na^+/H^+ exchange [111] and Na^+/K^+ ATPase [114] to achieve regulatory cell volume increase. The inhibition of ion channels at the cell membrane [111] prevents the loss of the ions thus accumulated. As a result, intracellular K^+ activity is enhanced and remains so for at least 16 hours [111]. Chronic exposure of the cells to hyperosmotic extracellular fluid leads eventually to accumulation of osmolytes [44].

Other

Mercury, cadmium and cobalt ions hyperpolarize the cell membrane of MDCK cells [49, 115–117]. The effect of mercury ions is apparently independent from calcium, that is, it occurs at mercury ion concentrations below those required to appreciably increase intracellular Ca^{2+} activity and it can be elicited in the nominal absence of extracellular Ca^{2+} [117]. In contrast, the effects of cadmium and cobalt ions are probably mediated by Ca^{2+}. In the nominal absence of extracellular Ca^{2+}, they lead to an only transient hyperpolarization, which can be elicited only once [115, 116]. This transient hyperpolarization is thought to result from a release of Ca^{2+} from cellular stores and subsequent transient activation of Ca^{2+} sensitive K^+ channels [115, 116].

Amphotericin B has recently been shown to activate a TEA inhibitable endogeneous K^+ channel in MDCK cells [118]. Little is known about the mechanism of activation. Furthermore, K^+ and Cl^- channels are apparently activated by mastoparan, a tetradecapeptide [119].

The biosynthesis and/or insertion of K^+ channels into the MDCK cell membrane is impaired by inhibition of protein synthesis with actinomycin D and cycloheximide, by inhibition of exocytosis with chloroquine, by destruction of the actin network with cytochalasin B and by depolymerization of microtubules with colchicine. It is not appreciably affected by tunicamycin, which inhibits glycosylation of nascent proteins. Chloroquine decreased cell membrane surface area and K^+ conductance in parallel, in line with the presumed exocytotic incorporation of channels and membrane into the cell surface. However, cytochalasin D did not significantly reduce and colchicine significantly enhanced the surface area of the cell membrane, pointing to a dissociation of cell membrane and channel trafficking [120].

MDCK cells transformed by a "bicarbonate shock" have been shown to display spontaneous oscillations of cell membrane potential, secondary to oscillations of cytosolic Ca^{2+} activity and subsequent activation of the Ca^{2+} sensitive intermediate K^+ channel [121–123]. The Ca^{2+} oscillations are inhibited by thapsigargin, pointing to pulsatile release of Ca^{2+} from cellular stores [124]. Similar oscillations of intracellular Ca^{2+}, K^+ channel activity and cell membrane potential have been observed in fibroblasts upon expression of *ras* oncogene [125], and it has been suggested that these oscillations are critical for cell proliferation [126, 127]. In MDCK cells, the activation of the K^+ channels are apparently required for migration of the cells [128].

Summary and conclusions

The MDCK cell has proven to be a useful model cell line for the study of properties and regulation of renal epithelial ion channels. Patch clamp studies disclosed the existance of several K^+ channels and of a Cl^- channel, and their regulation by hormones, cell volume, trace elements and drugs. Most hormones affect K^+ channels at least in part by increasing cytosolic Ca^{2+}. However, indirect evidence points to additional mechanisms contributing to K^+ channel activation. Cell swelling activates both K^+ channels and unselective anion channels. I_{Cln}, a protein cloned from

MDCK cells, is either a Cl^- channel or a regulator of thereof. I_{Cln} is up-regulated by cellular acidification and is crucial for rapid regulatory cell volume decrease.

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