inhibition in ClC-7 function. Leisle L, Ludwig CF, et. al recently utilized a method to robustly redirect ClC-7 to the plasma membrane by mutating lysosomal sorting motifs at the amino terminus of ClC-7 and by co-expressing an essential auxiliary subunit called Ostm-1 (Leisle, Ludwig, et al, 2011 EMBO J, 30,2140). using this strategy, we describe extracellular $[H^+]$-dependent inhibition of ClC-7 mediated ionic current in comparison with published data for ClC-5 from experiments by Picollo, Malvezzi, and Accardi (2010, JGP, 135, 653). In the tested conditions, ClC-7 is more sensitive than ClC-5 to extracellular H^+ (pK(0 mV) = 7.9 estimated for ClC-7/Ostm-1 and pK(0 mV) = 6.6 reported for ClC-5 expressed in oocytes). Further, we evaluate effects of increasing extracellular $[H^+]$ and varying intracellular $[Cl^-]$ on voltage-dependent transient current intrinsic to a transport deficient mutant of ClC-7 (E314A). Increasing $[H^+]_{ext}$ shifts the half-activating voltage of voltage-dependent charge movement in ClC-7 (mean $V_{1/2} \geq +20$ mV shift per pH unit increase), but does not drastically change the apparent charge valence (mean z = 0.85 to 0.95 q_e at all $[H^+]_{ext}$ tested). This result suggests that the voltage-dependent transient current does not arise from protonation of ClC-7, but that extracellular pH changes the work required for the electric field to elicit charge movement.

3228-Pos Board B383

Glialcam Affects CLC-Chloride Channels by Activating the Slow Gate

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Recently we have shown that GlialCAM, a cell adhesion molecule expressed mainly in glia, is a CLC-2 chloride channel subunit (1). Co-expression of CLC-2 with GlialCAM leads to a dramatic activation of currents and to a clustering of CLC-2 at cell contacts (1). Here we present an analysis of the interaction of GlialCAM with CLC-0 and CLC-1, two other CLC channels. CLC-1 biochemically interacts with GlialCAM and GlialCAM induces clustering of CLC-1 at cell contacts. Currents of CLC-1 and CLC-0 are modified by GlialCAM, but our study was focused mainly on CLC-0 because here slow and fast gating can be more easily separated. In particular, co-expression with GlialCAM led to an increase of the residual open-probability of the slow gate at positive voltages and the block by Zinc, which is mediated by affecting the slow gate (2) was largely reduced. In addition, deactivation of the slow gate was dramatically slowed, but retained the typical large temperature dependence (3). The mutant C212S, which lacks slow gating transitions (4), was not affected by co-expression with GlialCAM. Thus, GlialCAM is able to interact with CLC channels different from CLC-2. Our results show that in the context of CLC-0, the interaction with GlialCAM activates the slow (common) gate, suggesting that a similar mechanism underlies the activation of CLC-2. (Supported by Compagnia San Paolo and Telethon Italy (grants GGP08064 and GGP12008).)

1) Jeworutzki et al. 2012 Neuron73:951.
2) Chen 1998 JGP 112:715.
3) Pusch et al. 1997 JGP 109:105.
4) Lin et al. 1999 JGP 114:1.

3229-Pos Board B384

Kidney CLC-K Chloride Channels Show Differential Pharmacological Profiles Depending on the Heterologous Expression System

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The human chloride channels CLC-Ka and CLC-Kb play a pivotal role in kidney by controlling chloride and water absorption. Both channels require barttin as an accessory subunit for full activity. Mutations in CLC-Kb and barttin genes lead to severe renal salt loss and CLC-K polymorphisms are associated with hypertension. Therefore, novel therapeutic approaches targeting CLC-K/barttin might be helpful in treating these diseases. We have recently identified relevant molecular determinants that distinguish CLC-K activators from blockers using the *X.laevis* oocyte expression system. We recognized niflumic acid (NFA) as a powerful activator and phenyl-benzofuran carboxylic acid analogs as potent inhibitors. Here, by using these molecules as lead compounds and the whole cell configuration of the patch-clamp technique, we explored the pharmacological profile of CLC-K/barttin expressed in mammalian HEK-293 cells. Similarly to what observed in amphibian oocytes, benzofuran derivatives resulted as efficacious blockers of CLC-Ka mediated currents. A rational drug design allowed us also to ameliorate drug potency, finally identifying a newly synthesized benzofuran derivative (SRA-36) with an inhibitory affinity of 4 μM. Surprisingly, NFA failed to increase CLC-Ka currents, producing only an inhibitory effect in the 1-1000 μM range. In order to gain insight into this relevant difference between the two expression systems, we are currently evaluating the NFA effect on mutants of CLC-Ka that drastically affect NFA potentiation in oocytes. Besides identifying new therapeutic drugs, our results could shed light on structural determinants of CLC-Ks channels activity. (MIUR- COFIN-2009; Telethon GGP08064, GGP10101, GGP12008).

3230-Pos Board B385

Electrophysiological Properties of Novel Mutations in CLC-1 Chloride Channel of Korean Patients with Myotonia Congenita

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Myotonia Congenita is a genetic disease that displays the symptom of muscle stiffness and impaired muscle relaxation caused by hyperexcitability of the plasma membrane. There are two types of Myotonia Congenita; Autosomal dominant Myotonia Congenital (Thomsen's disease) and autosomal recessive generalized Myotonia (Becker's myotonia) are caused by mutations in the skeletal muscle chloride channel, ClC-1.

ClC-1, the member of a large family of anion channels, is voltage-gated chloride channel. It is abundantly expressed in human skeletal muscle. In skeletal muscle, the voltage-gated chloride channels contribute to stabilize the resting membrane potential and control electrical excitability. ClC-1 channels contain double-barreled structure which consists of two identical protopores. Each pore is voltage dependent and functions independently.

When mutations in the gene for ClC-1 underlie Myotonia Congenita, it can affect normal function of the channel and damage the specialized property of independently working double pores. Since Mytonia Congenital was first diagnosed, a number of mutations widely displayed in the protein have been revealed so far. Here, nine mutants (p.M128I, p.S189C, p.M373L, p.P480S, p.G523D, p.M609K, p.T310M, p.R317X, p.R47W, A298T and p.G355R) from Korean patients who suffer from Myotonia Congenita were reported in 2009. We studied the functional changes of each mutant by using patch clamp method. We observed remarkably reduced chloride conductance from most of mutants. Mutants, p.M128I, and p.G523D showed steady-opened current pattern compared to WT. Furthermore, open probability of mutants was slightly or markedly altered and this clearly indicates the modification of pore property.

3231-Pos Board B386

Purification and Functional Reconstitution of the TMEM16A Ca^{2+} Activated Cl^- Channel

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Ca^{2+}-activated Cl^- channels (CaCCs) play numerous physiological roles ranging from electrolyte secretion in epithelia and glands, to muscle contraction, olfactory transduction and nociception. Despite their physiological importance the molecular identity of these proteins remained elusive until recent work showed that TMEM16A and B, two members of the TMEM16 family of membrane proteins, are critical components of CaCCs. Heterologous expression of these two genes in oocytes and cells results in currents closely resembling native CaCCs. It is however unknown whether these proteins alone are necessary and sufficient to form functional Ca^{2+} activated Cl^- channels, or whether association to other subunits is required. For example, neither TMEM16A nor B bear any conventional Ca^{2+} binding motifs and studies suggest that association of a TMEM16A isoform with Calmodulin is required for Ca^{2+} sensitivity. To investigate whether TMEM16A alone forms functional CaCCs or if association to other partner proteins is required we expressed, purified and reconstituted TMEM16A in proteoliposomes. We found that purified TMEM16A mediates Ca^{2+} dependent Cl^- fluxes with an apparent Km of ~300 nM, a value comparable to that measured in patch clamp experiments. Channel opening is also promoted by the application of high positive voltages, consistent with electrophysiological measurements. Channel activity is diminished by CaCC inhibitors such as Niflumic Acid, NPPB, NPA and DIDS with $K_{1/2}$'s comparable to those measured for native and heterologous CaCCs. Mutating two conserved glutamates in the TM5-6 intracellular loops abolishes Ca^{2+} sensitivity of the fluxes, in a manner similar to what was reported for heterologously expressed TMEM16A.

In conclusion, our results demonstrate that the purified TMEM16A protein recapitulates all the fundamental biophysical and pharmacological properties of native and heterologously expressed CaCC currents indicating that association with other partner proteins is not strictly required for function.