Summary

• A robust assay of ClC-1 with $Z'$ constantly $>0.5$
• Biophysical and pharmacological characteristics as expected for ClC-1
• Qube offers the possibility to apply compounds both intra- and extracellularly
• Advanced analysis of compound effects on channel kinetics are available at a few clicks

Introduction

The family of CLC proteins comprises both chloride channels and transporters that are expressed in a great variety of tissues. Subtype 1 of the CLC family (ClC-1) constitutes a voltage-gated chloride channel that is predominantly expressed in skeletal muscle fibers. In these cells, ClC-1 channels are key players in repolarizing the membrane potential and propagating action potentials that eventually regulate muscle contraction. Consequently, it is not surprising that malfunction of the channel is related to disease states. For example, the hereditary disease myotonia that is characterized by muscle membrane hyperexcitability was linked to mutations in the CLCN1 gene that codes for ClC-1 (Miryounesi, Ghafoori-Fard, & Fardaei, 2016). Aside from its relevance in muscle cells, a recent study revealed a role of ClC-1 in epilepsy (Chen et al., 2013).

ClC-1 has a complex gating, channels are time- and voltage-dependently activated at positive potentials and the channel is strongly inwardly rectifying. ClC-1 has two distinct gating mechanisms that are governed by a fast (protopore) and a slow (common) gate. The fast gate is about 10-fold faster than the common gate, a fact that has often been exploited by scientists to study the two gates individually. The channel is further sensitive to pH, anions and intracellular ATP (Jentsch, 2015).

Albeit ClC-1 has been subject to many investigations over the past three decades, its pharmacology is only poorly developed with few moderately selective inhibitors and activators available at the moment. Many pharmacological tool agents targeting ClC-1 bind to the channel from the cytosolic side. This is not a problem as long as the compound is sufficiently membrane permeable. The classical ClC-1 inhibitor 9-anthracen carboxylic acid (9-AC) is highly lipophilic and hence passes the plasma membrane with ease. A fact that allows extracellular application of the compound in a patch clamp experiment. However, possible new compounds that also bind to ClC-1 from the cytosolic side may show different characteristics. Therefore, an assay that is limited to compound application to the external side may result in false negatives. Qube offers the possibility to exchange both extracellular and intracellular solution during an experiment making it well suited to study ClC-1 pharmacology.

In this report, we show a ClC-1 assay on Qube that shows biophysical characteristics as expected for ClC-1. We further introduce Sophion’s Analyzer software that allows rapid analysis of large data sets to answer advanced electrophysiological questions, in the present case: What is the mode of action of a novel, unknown compound?

Results and discussion

To characterize ClC-1 biophysically, we applied a series of voltage steps to the cells in the whole cell configuration. Typical current response traces are shown in Figure 1A. The respective current-voltage relationships of the instantaneous (red) and steady state current (black) are shown in Figure 1B. A large inward current was recorded instantaneously, representing the current passing through all channels open at +60 mV. Due to the voltage sensitive nature of ClC-1, almost no inward current was detected in the steady state.
Different pharmacological tool agents are available that either act as gating modifier (i.e. shift the equilibrium between open and closed channels towards more depolarized potentials) or as pore blocker. ClC-1’s intimate relationship between permeability and gating suggests that any pore blocking agent will also modify ClC-1 gating i.e. shift the half-activation potential ($V_{1/2}$) towards more positive potentials. 9-AC acts as pore blocker and was shown to bind to the pore from the intracellular side (Estevez, Schroeder, Accardi, Jentsch, & Pusch, 2003).

Figure 2 shows the effect of intracellularly applied 9-AC (100 µM) on ClC-1 conductance. Almost no current was mediated through ClC-1 channels over the entire voltage spectrum following 9-AC exposure (Figure 2A). Analysis of the inactivation curve (Figure 2B) revealed that the compound in fact acts predominantly as pore blocker as even at +100 mV, almost no tail currents were detected.

We used a compound (Compound X) that is known to act as gating modifier to demonstrate the assay’s capability to differentiate between pore block and gating modification. To assess the compound’s effect on ClC-1 gating, a set of voltage steps from -140 mV to +120 mV in 20 mV increments was applied to the cells and tail currents at -100 mV were used to generate deactivation curves (Figure 3A). The half activation potential ($V_{1/2}$) in the control condition was calculated to $V_{1/2}$(Control) = -4 mV. This value is more positive than values reported in the literature as the used internal solution contained a relatively low concentration of Cl- and $V_{1/2}$ strongly depends on ionic strength (Accardi & Pusch, 2000). Exposure to Compound X (500 µM) caused a shift in $V_{1/2}$ by 27 mV to $V_{1/2}$(Compound X) = +23 mV. To further investigate the mode of action of compound X, we recorded a dose response curve both on tail current amplitude and on deactivation kinetics represented.
by Tau of a monoexponential fit to the tail currents (Figure 3B and C) (see methods). Compound X exhibited a ~5-times higher potency on deactivation kinetics (Figure 3C) compared with open channel inhibition (Figure 3B). This suggests that the compound stabilizes the closed state of CiC-1 rather than inhibiting the conducting pore.

Finally, we validated the assay using Z’ analysis. Z’ values were determined from a column with 0.1% DMSO control versus a column with internally applied 100 µM 9-AC. Z’ was > 0.5 in 7 individual experiments (Figure 4).

Fig. 3: Compound X inhibits CiC-1 currents by modulating channel gating. A: Representative inactivation curve recorded at the tail current in the presence (V_{1/2}(9-AC) = + 23 mV) and absence (V_{1/2}(Control) = - 4 mV) of cytosolic compound X (500 µM). B: Noncumulative dose response curve obtained from the tail current amplitude of a -20 mV pre-pulse and normalized to control period and full block period (n = 6-9 per concentration). C: Noncumulative dose – dependent effect of compound X on CLC-1 deactivation kinetics represented by Tau (Monoexponential fit to the tail current of a +120 mV pre-pulse).

Fig. 4: Z’ values were calculated from normalized current inhibition values obtained from column 23 (0.1% DMSO control) and column 24 (9-AC 100 µM applied intracellularly) of a 384 – well plate.

Conclusion

As illustrated above by means of the challenging CiC-1 assay, Qube offers a high degree of flexibility in the assay design. In addition to this, the related Qube software, Analyzer, makes it very easy to rapidly set up advanced analyzing templates that, once generated, can be applied to every newly generated data. Taken together this makes Qube an ideal platform for automated high-throughput patch clamp studies.
Methods

Cells and Cell Culture

CHO cells stably expressing hClC-1 were provided by ChanTest. Cells were cultured following the manufacturer’s guidelines and harvested according to Sophion’s standard procedure for CHO cells including Trypsin.

Electrophysiology

Whole cell patch clamp recordings were performed on Qube following Sophion’s standard procedure using multi hole QChips (10 holes per well). ClC-1 currents were elicited using a 100 ms long voltage pre step to +60 mV followed by a series of voltage steps (300 ms) ranging from -140 mV to +120 mV in 20 mV increments. Tail currents were analyzed at -100 mV.

References:


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