SOPHION BIOSCIENCE

Application Report

The Intermediate Conductance Ca^{2+} - Activated K^+ channel on Qube 384

A robust assay well-suited for a screening campaign

Summary

- $K_{Ca}3.1$ (IK, SK4, $IK_{Ca}1$, IK-1 or $K_{Ca}4$) encoded by KCNN4 is an emerging target in vascular inflammation, asthma, autoimmune diseases as well as different types of cancer
- We developed a $K_{Ca}3.1$ assay with high DMSO tolerance, no apparent run down and Z` values well above 0.5
- The assay does neither rely on an internal solution exchange nor on F^- in the internal solution.
- Both biophysical and pharmacological properties are in good agreement with literature.

Introduction and scope

Ca^{2+}-activated K^+ channels (K_{Ca}) are integral players in setting the membrane potential and regulating Ca^{2+} homeostasis. The human genome consists of a total of 8 K_{Ca} channels that differ in their unitary conductance, genetic relation to each other and mechanism of Ca^{2+} binding. The channels are subdivided in two groups according to the aforementioned characteristics. The first group was initially characterized by their structural similarity and comprises of $K_{Ca}1.1$ (BK), $K_{Ca}4.1$, $K_{Ca}4.2$ and $K_{Ca}5.1$. Later studies revealed that these channels have in fact a different activation mode and only $K_{Ca}1.1$ (BK) is a truly Ca^{2+}-activated channel aside from its voltage-dependence. The second group is characterized by their unitary conductance and encompasses three small conductance (SK) channels $K_{Ca}2.1$ (SK1), $K_{Ca}2.2$ (SK2) and $K_{Ca}2.3$ (SK3), and an intermediate-conductance channel $K_{Ca}3.1$ that is also known as SK4, $IK_{Ca}1$, IK-1 or $K_{Ca}4$ encoded by KCNN4 (Wei et al., 2005). $K_{Ca}3.1$ was cloned independently by three groups in 1997, therefore, the channels biophysical and pharmacological properties are already well-established (Wulff & Castle, 2010).

$K_{Ca}3.1$ is tightly regulated by intracellular Ca^{2+} via a Ca^{2+}-calmodulin-dependent mechanism but insensitive to changes in membrane potential. The EC_{50} values for Ca^{2+} activation have been reported from 95–350 nM, which is very similar to those of SK channels. The channels ion selectivity follows a type III or IV Eisenmann sequence with K^+ (1.0) = Rb^+ > NH_4^+ > Cs^+ >> Na^+, Li^+, $NMDG^+$. The unitary conductance of $K_{Ca}3.1$ ranges from ~32–39 pS (symmetrical K^+ gradient). The IV relationship shows inward-rectification at positive potentials, which can be attributed to voltage-dependent open channel block of the by intracellular Ca^{2+} and Mg^{2+} ions (Ishii et al., 1997; Ledoux, Bonev, & Nelson, 2008)

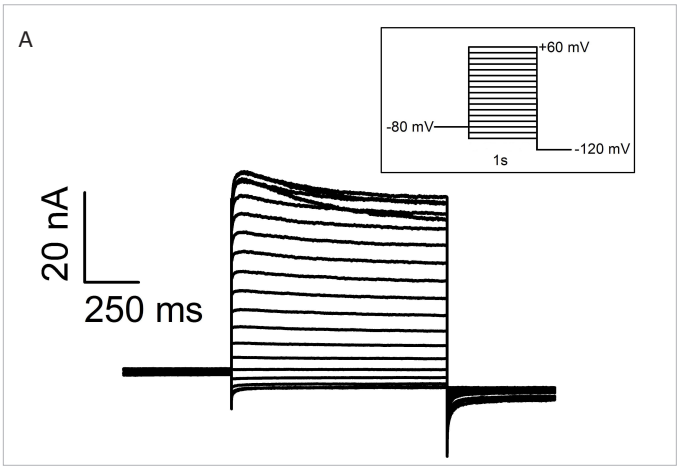
$K_{Ca}3.1$ is expressed in a large diversity of tissues in mammals but is, with a few exceptions, found to be absent from excitable tissues such as cardiac myocytes and skeletal muscles. Most prominent expression of the channel is found in tissues involved in fluid and salt secretion such as the colon and salivary glands as well as in cells of the hematopoietic system (Ishii et al., 1997).

In many cells, $K_{Ca}3.1$ is pivotal in regulating cell proliferation, migration and cytokine synthesis, thus dysregulation or overexpression of the channel is tightly linked to some disease states. In the past two decades much research has been focused on the role of $K_{Ca}3.1$ in autoimmune diseases. In activated lymphocytes, macrophages as well as mast cells, $K_{Ca}3.1$ overexpression was linked to imbalanced migration and proliferation, often resulting in an over amplified or lacking immune response (Ghanshani et al., 2000). The channel was further reported to play a central role in vascular inflammation, asthma and tumorigenesis (Bonito & Sauter, 2016; Bradding et al., 2009; Chou et al., 2008).

Results and discussion

In order, to validate the assay biophysically, we recorded current traces in response to rectangular voltage pulses of different potentials (Fig. 1A). In line with the literature, current traces exhibited inward rectification at positive potentials in a time-dependent manner; this becomes even more apparent in the steady state IV relationship (Fig.1B). This inward rectification is due to the presence of divalent cations in the internal solution that voltage-dependently blocks the channel. The slight outward rectification at negative potentials is a result of an asymmetrical K^+ ion gradient.

We assessed the stability of the assay when challenged with different concentrations of DMSO. Figure 2 shows the current over time (IT) plot of all wells that were exposed to 0.02% DMSO. No significant run-down was observed during a 4 min test period (Table1). As expected, application of the $K_{Ca}3.1$ – specific tool agent, NS6180 (1 µM), at the end of the experiment completely inhibited the current. Increasing DMSO concentration to 0.3 and 0.5% did not affect assay stability (Table1). This fact makes the assay particularly well-suited for a screening campaign as it allows for the use of higher test concentrations in case the concentration of the stock solution is fixed. Employing the method described by Zhang et al. (1999), we calculated the Z` values for the respective DMSO concentration (Tab.1). All Z` values were far above the quality threshold of 0.5, highlighting the outstanding robustness of the assay.



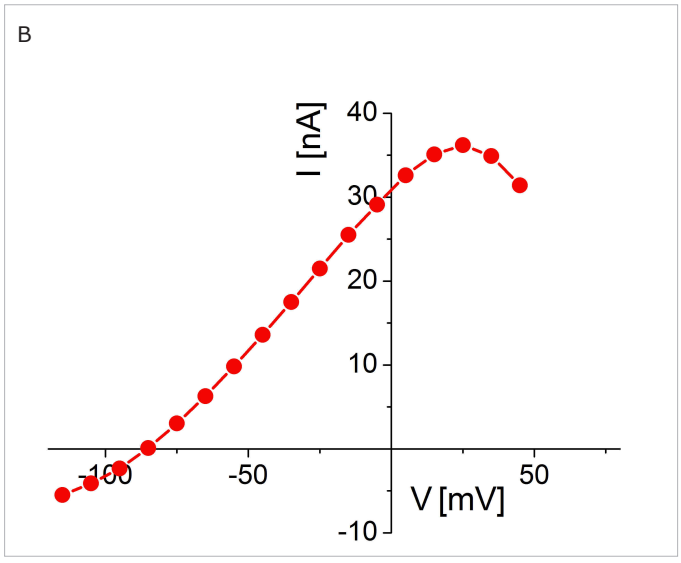


Fig. 1: A: Representative current traces evoked by a family of voltage steps as shown in the insert. The data were recorded using 1 µM free Ca^{2+} (calculated) in the internal solution and a multi-hole QChip. B: Steady state IV curve recorded at the end of the 1s long pulse. Data were corrected for the measured liquid junction potential.

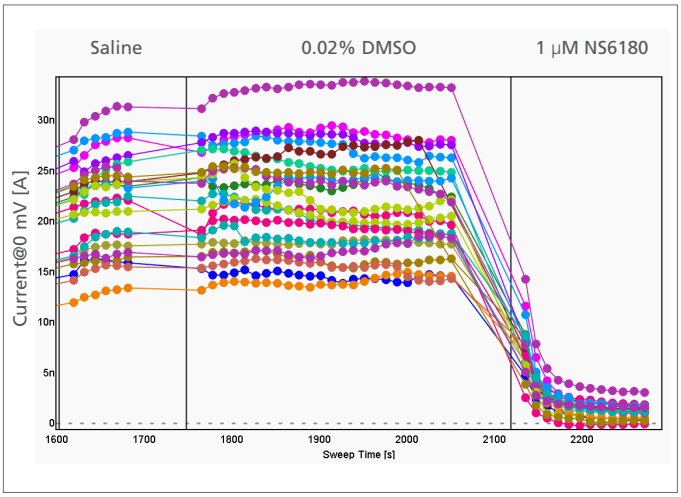
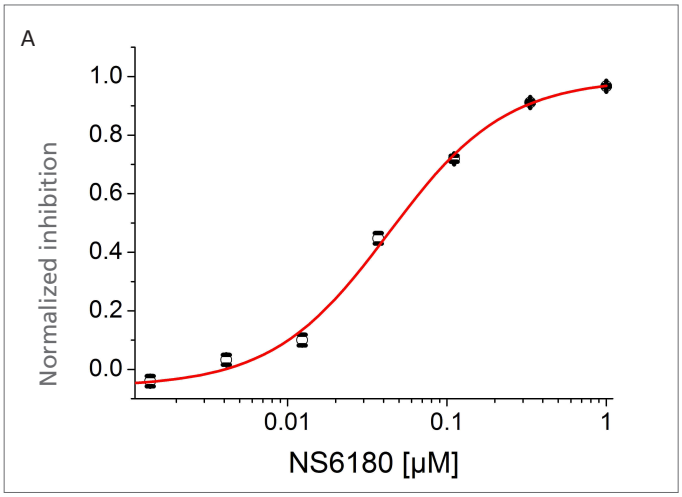


Fig. 2: Current over time (IT) plots of the 0.02% DMSO group. Each line represents one measurement site of a multi-hole QChip. Every 10 s, a voltage ramp of 1 s duration, ranging from -100 to +60 mV, was applied to the cell membranes. Shown current values were determined at 0 mV.

Table 1: Summary of quality data recorded in different concentrations of DMSO. Run down and Z' values were determined at the end of a 4 min test period using a multi-hole QChip. Shown are mean ± SD.

DMSO	0.02%	0.30%	0.50%
Run down [%/min]	1±3	0±2	0±3
Z' value	0.64	0.79	0.67
Success rate [%]	72	66	78



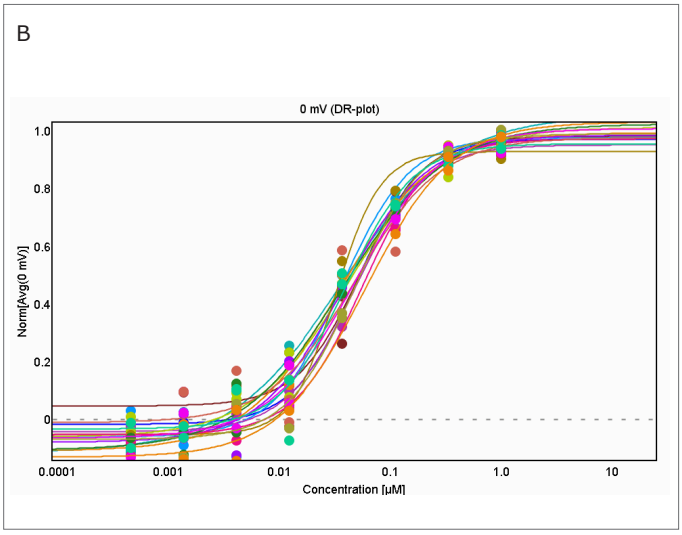


Fig. 3: Non-cumulative dose response curve of NS6180 recorded using 1 µM free Ca^{2+} (calculated) in the internal solution. A: Data represents mean ± SEM of n = 18-28 sites of a multi-hole QChip. A Hill function was fitted to the data with IC_{50} = 43 nM and a Hill parameter of 1.2. B: Each individual row of a 384 compound plate was used to generate single dose response curves. As apparent from the low data spread and lack of DMSO sensitivity, one could also record IC_{50} values of at least 24 different compounds in one experiment.

We used NS6180 to validate the assay pharmacologically. Cells were subjected to 7 different concentrations of NS6180 in a non-cumulative fashion followed by a supramaximal dose of 1 µM NS6180 at the end of the experiment. Current inhibition was evaluated as relative to baseline and supramaximal dose period. Figure 3A shows the mean dose response relationship obtained across the whole plate. The calculated IC_{50} value was 43 nM. This value correlates well with values reported in the literature (Jenkins et al., 2013; Strøbæk et al., 2013). The slightly lower potency can likely be attributed to the shorter compound exposure time.

It is not practical to use a whole 384-well QChip to determine the IC_{50} value of just one single compound. Therefore, we tested the reproducibility of the assay using just n=2 per concentration to determine the IC_{50} value. The compound plate was set-up with one concentration of NS6180 in each two columns and the remaining columns were used for different DMSO controls as shown in Figure 2 and Table 1. Figure 3B shows 16 individual Hill functions fitted to data recorded in one QChip row and the respective IC_{50} values can be seen in Table 2. As apparent from the very low data spread (IC_{50},min /IC_{50},max = 1.8) the assay shows a very high degree of accuracy and considering the absence of DMSO sensitivity, this would allow for simultaneous determination of IC_{50} values of at least 24 different compounds.

Table 1: Summary of IC_{50} values obtained using n=2 per concentration as shown in Fig.3B. "Fit quality" represents a measure of the goodness of the fit with "0" being the ideal fit. All IC_{50} values lie very close to each other allowing to record IC_{50} values of at least 16 compounds in one experiment.

	Fit quality	IC_{50}[nM]
Group NS6180; ROW 1	0.13	50
Group NS6180; ROW 2	0.12	36
Group NS6180; ROW 3	0.09	37
Group NS6180; ROW 4	0.15	55
Group NS6180; ROW 5	0.09	48
Group NS6180; ROW 6	0.10	34
Group NS6180; ROW 7	0.16	42
Group NS6180; ROW 8	0.18	38
Group NS6180; ROW 9	0.18	33
Group NS6180; ROW 10	0.13	56
Group NS6180; ROW 11	0.34	49
Group NS6180; ROW 12	0.12	38
Group NS6180; ROW 13	0.13	48
Group NS6180; ROW 14	0.13	48
Group NS6180; ROW 15	0.18	40
Group NS6180; ROW 16	0.12	59

Conclusion

In conclusion, we developed an automated patch clamp assay for $K_{Ca}3.1$ on Qube that exhibits a very high degree of reproducibility and an outstanding stability.

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