

Pharmacological analysis of heterologous and endogenous expressed Kv1.3 channels in Sf21 insect cells and T-lymphocytes

Author: Maike Hellmers, M.Sc., Electrophysiologist, *conoGenetix biosciences GmbH*, Munich.

Summary

The voltage-gated potassium channel Kv1.3 is expressed in human naive, central memory T cells (T_{CM}), and effector memory T cells (T_{EM}). T_{EM} cells are important mediators of a variety of chronic inflammatory autoimmune diseases including multiple sclerosis (MS), rheumatoid arthritis (RA), psoriasis, and vasculitis. Antigenic stimulation of T_{EM} cells results in upregulated Kv1.3 channel expression.

It is proposed that a selective block of Kv1.3 channels leads to an inhibition of functionality of T_{EM} cells without affecting the function of naive and T_{CM} cells. Thus, Kv1.3 is a promising therapeutic target that could be used to efficiently medicate chronic autoimmune diseases without generating typical side effects associated with today's immunosuppressive therapies.

In the laboratory of *conoGenetix biosciences* the insect cell line *Spodoptera frugiperda* (Sf21) is used in combination with a Baculovirus infection system for the heterologous expression of recombinant ion channels. Sf21 cells offer highly specific protein expression and correct posttranslational modifications resulting in functional channels with comparable electrophysiological parameters of ion channels *in vivo*.

In this *Application Note* we compare the electrophysiological properties of the potassium selective ion channel Kv1.3 expressed in Sf21 cells and endogenously expressed Kv1.3 channels in T_{EM} cells. For the pharmacological analysis the 4-channel Nanion Patchliner® and one of our innovative, Kv1.3 specific, proprietary peptides was used.

Results

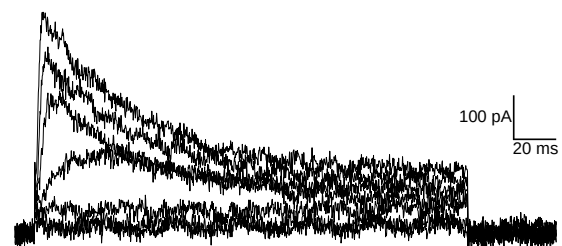
For the comparison of characteristic voltage dependent Kv1.3 channel activation a typical voltage protocol from -60 mV to +60 mV in 20 mV steps (activation pulse 200 ms) was used. Cells were clamped at a holding potential of -100 mV.

Figure 1 shows the current response of two individual Sf21 and T_{EM} cells (figure 1A and 1B, respectively) and the pulse protocol for voltage dependent Kv1.3 activation (1C).

A) Sf21 cell



B) T_{EM} cell



C) Pulse Protocol

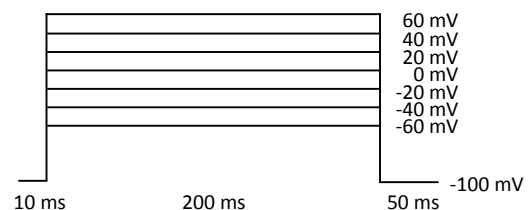


Figure 1:

Representative voltage dependent activation of Kv1.3 channels expressed in Sf21 cells (A) and endogenously expressed in T_{EM} cells (B). (C) Pulse protocol for voltage dependent Kv1.3 activation.

Application Note

Channel:

Kv1.3

Cells:

Sf21 and Effector Memory T cells

Tools:

Patchliner®

Figure 2 shows the corresponding normalized voltage-current-correlation. Kv1.3 starts to open at a depolarization of the membrane from the holding potential (-100 mV) to -20 mV and reaches its maximum open state at +60 mV. Even though the expression of Kv1.3 is much stronger in Sf21 cells, the characteristic voltage dependent activation remains unchanged.

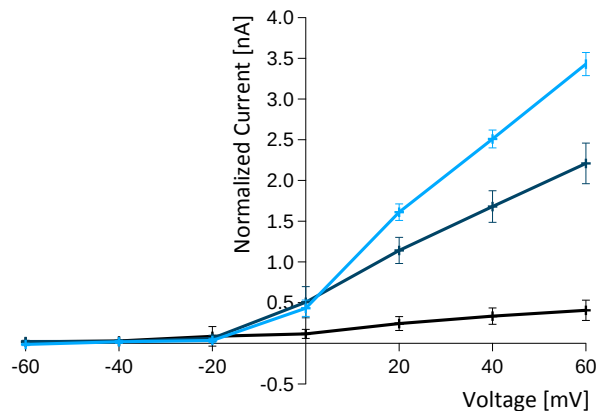


Figure 2:

Characteristic normalized voltage-current-correlation of Kv1.3 channels expressed in Sf21 (dark-blue and light-blue lines) and T_{EM} cells (black line); n= 41, n= 12 and n=29, respectively. With the Sf21 Baculovirus expression system it is possible to express high amounts of Kv1.3 potassium channels. According to the infection rate Sf21 cells can adapt different expression levels.

The maximal observed current, which clearly originates from Kv1.3 activity, and the applied voltage allow to assess the total conductivity of all Kv1.3 channels. Accordingly the number of channels can be extracted using the known conductivity of a single Kv1.3 channel (13 pS; Grissmer et al., 1990). The maximal K⁺ outward current is defined as the difference between activated unblocked and maximally blocked (full block) currents during the first 20 % of the current trace. Due to the infection conditions, it is possible to control the expression level of Kv1.3 in Sf21 cells, according to the needs of the investigation. Under our standard conditions a maximal current of 2 - 3 nA, a conductance of 33 - 50 nS and a number of 2500 - 3800 channels per cell can be estimated.

It is postulated that quiescent human peripheral blood naive, T_{CM}, and T_{EM} cells have similar channel phenotypes expressing ~300 voltage-gated Kv1.3 potassium channels per cell (Matheu et al., 2008). The patch clamp analysis displayed that purified and anti CD3 activated T_{EM} cells showed an average maximal current of 290 pA, a conductance of 4,8 nS and consequently a calculated number of 370 channels per cell (mean values from n=35 cells).

One should note that the calculations are made on the presumption that all present Kv1.3 channels are activated and opened by the applied depolarization. In addition the conductance of the cell membrane and subconductances were not taken into account.

Application Note

Channel:

Kv1.3

Cells:

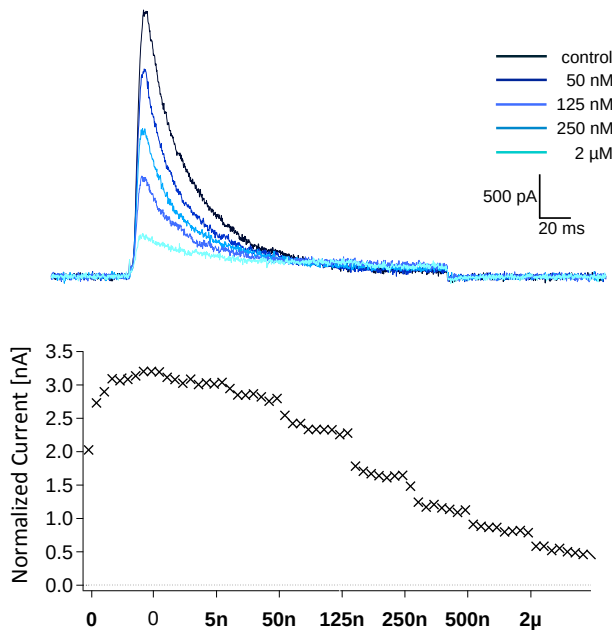
Sf21 and Effector Memory T cells

Tools:

Patchliner®

The highly specific Kv1.3 block was performed with a new and proprietary peptide compound designed by *conoGenetix*. The peptide shows high affinity to Kv1.3 channels as shown in figure 4.

A) Sf21 cell



B) T_{EM} cell

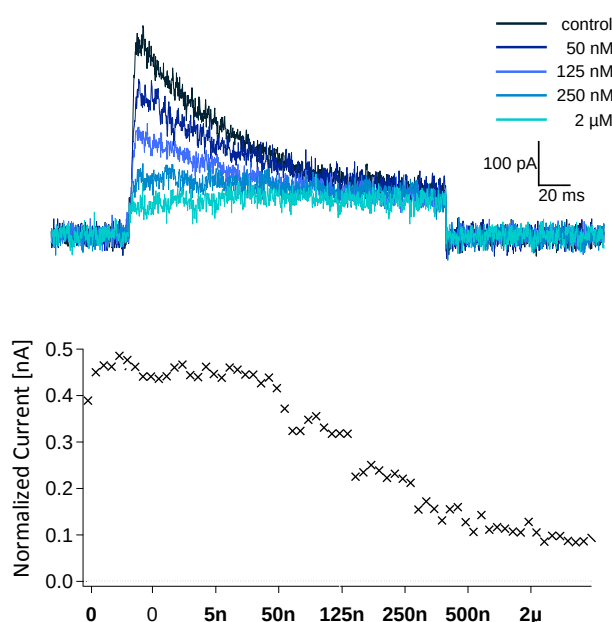


Figure 4: Block of Kv1.3 channels expressed in Sf21 (A) and T_{EM} (B) cells. Representative raw current traces and time dose responses in the presence of increasing concentrations of the Kv1.3 antagonistic peptide.

As shown in figure 5 the averaged IC₅₀ value of the peptide for Kv1.3 channels expressed in Sf21 and T_{EM} cells is in good agreement with 91,1 nM ± 4,0 nM on Sf21 and 92,8 nM ± 2,3 nM on T_{EM} cells.

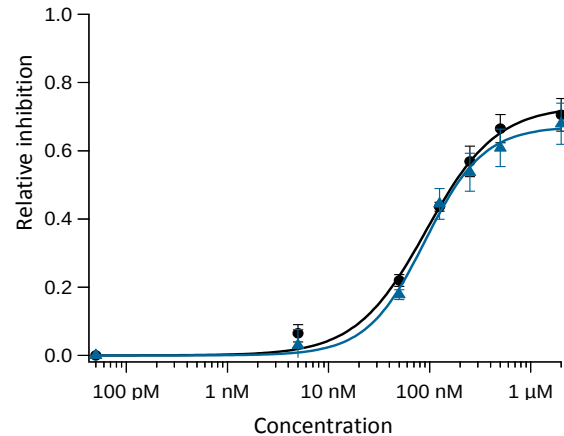


Figure 5:

Dose response curves of the specific peptide on Kv1.3 channels. Blue line Sf21 cells, IC₅₀ = 91,1 nM ± 4,0 nM (n=4); black line T_{EM} cells, IC₅₀ = 92,8 nM ± 2,3 nM (n=4).

Conclusion

The heterologous expression system in Sf21 cells displays a promising method for electrophysiological analysis of pharmacologically interesting ion channels *in vitro*. In our laboratory we were already able to overexpress different potassium channels of the Kv1 subfamily in Sf21 cells. Based on our observations it can be assumed that the folding and insertion of Kv1.3 ion channels into the plasma membrane of Sf21 cells is comparable to native proteins in T_{EM} cells.

We have clearly demonstrated that heterologously expressed Kv1.3 channels can serve as a model system to study drug targeting by assessment of electrophysiological parameters. This established model system would allow to furthermore investigate the effect of e.g. channel mutants and expression levels on drug effectiveness.

Application Note

Channel:

Kv1.3

Cells:

Sf21 and Effector Memory T cells

Tools:

Patchliner®

Methods

Cells

The *Spodoptera frugiperda* (Sf21) insect cell line was purchased from Invitrogen.

Untouched T_{EM} cells from quiescent human peripheral blood were purified using Rosette Separation and StemCell Negative Selection columns.

Cell culture

Sf21 suspension roller cultures were cultured, infected and harvested according to the conoGenetix biosciences standard cell culture protocols.

Purified T_{EM} cells were cultured according to our specific cultivation protocols and Kv1.3 expression was induced with anti CD3 activation.

Electrophysiology

Whole cell patch clamp recordings were performed according to our standard patch clamp protocols with the 4 - channel Nanion Patchliner®. Due to the diameter of the cells for Sf21 cells a 2-3 MΩ resistance, for T_{EM} cells a 5-6 MΩ resistance NPC-16 chip was used. The patch clamp solutions were adjusted to the Kv1.3 potassium channel and the used cell line. The pulse protocol for the comparison of the voltage-current-correlation was identical for both cell lines (see figure 1C).

For pharmacological investigations the Kv1.3 currents were elicited using a voltage step from the holding potential (-100 mV) to +60 mV for 200 ms and back to the holding potential. Voltage protocols were elicited every 10 s (KvIV) and 20 s (KvPharm).

The detected currents were pre-amplified on headstages and digitized, using a HEKA *quadro patch clamp amplifier EPC10*. Data recording was conducted using the PatchMaster software (HEKA Elektronik Dr. Schulze GmbH, Lambrecht / Pfalz, Germany). Data analysis and presentation was realized using the softwares IGOR Pro Version 6.22A (Wave Metrics Inc., USA) and Microsoft Excel 2010 (Microsoft Corporation, USA).

Please contact us for further information.

References

1. Grissmer, S., Dethlefs, B., Wasmuth, J. J., Goldin, A. L., Gutman, G. A., Cahalan, M. D., Chandy K. G. (1990). Proc. Natl. Acad. Sci USA, 87, 9411-9415
2. Matheu, M. P., Beeton, C., Garcia, A., Chi, V., Rangaraju, S., Safrina, O., Monaghan, K., Uemura, M. I., Li, D., Sukumar, P., de la Maza, L. M., Monuki, E., Flügel, A., Pennington, M. W., Parker, I., Chandy, G., Cahalan, M. D. (2008). Immunity, 29, 602–614



conoGenetix biosciences GmbH
Dr. Andreas Klostermann, CEO
Am Klopferspitz 19
82152 Planegg OT Martinsried

Telephon +49-(0)89-54726950
Fax +49-(0)89-54726959
Internet www.conogenetix.de