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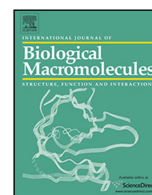

Dromedary immune response and specific Kv2.1 antibody generation using a specific immunization approach

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ABSTRACT

Voltage-gated potassium (Kv) channels form cells repolarizing power and are commonly expressed in excitable cells. In non-excitable cells, Kv channels such as Kv2.1 are involved in cell differentiation and growth. Due to the involvement of Kv2.1 in several physiological processes, these channels are promising therapeutic targets. To develop Kv2.1 specific antibody-based channel modulators, we applied a novel approach and immunized a dromedary with heterologous *Ltk-* cells that overexpress the mouse Kv2.1 channel instead of immunizing with channel protein fragments. The advantage of this approach is that the channel is presented in its native tetrameric configuration. Using a Cell-ELISA, we demonstrated the ability of the immune serum to detect Kv2.1 channels on the surface of cells that express the channel. Then, using a Patch Clamp electrophysiology assay we explored the capability of the dromedary serum in modulating Kv2.1 currents. Cells that were incubated for 3 h with serum taken at Day 51 from the start of the immunization displayed a statistically significant 2-fold reduction in current density compared to control conditions as well as cells incubated with serum from Day 0. Here we show that an immunization approach with cells overexpressing the Kv2.1 channel yields immune serum with Kv2.1 specific antibodies.

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1. Introduction

Voltage-gated potassium (Kv) channels are known to create the repolarizing power of excitable cells that terminates the action potential. The Kv2.1 channel is a mammalian orthologue of the shab-family of Kv channels and underlies one of the predominant delayed rectifier K^+ currents in mammalian central nervous system neurons [1]. Besides their role in cell excitability, Kv2.1 channels are also involved in regulating insulin secretion in β-cells and in the differentiation and growth of non-excitable cells [2]. Accordingly, Kv2.1 channels have been reported to be overexpressed in at least uterine [3] and gastric tumorcells [4,5]. Several reports indicate that the inhibition of these K^+ currents suppresses the proliferation of cancer-type cells [3–5]. Given the widespread role of Kv2.1 channels in multiple physiological processes, they are considered to be important therapeutic targets for treatment of neurological and cardiovascular disorders, type 2 diabetes and suppression of cancers. Several Kv2.1 selective peptide blockers have been isolated from animal venoms. In particular, Hanatoxin 1 and Hanatoxin 2, isolated from the venom of Chilean *Tarantula* [6], and Ssmtx-1 isolated from the venom of a centipede [7]. However, because of their venom origin, toxins could have adverse effects and their safe therapeutic application must be demonstrated [8]. Therefore, it is more interesting to isolate a non-toxin Kv2.1 modulator, which is able to reduce the K^+ currents. Antibodies that inhibit the channel from the intracellular side have already been reported [9,10]. To the best of our knowledge, antibodies operating via extracellular regions and modulating the Kv2.1 channels have never been described.

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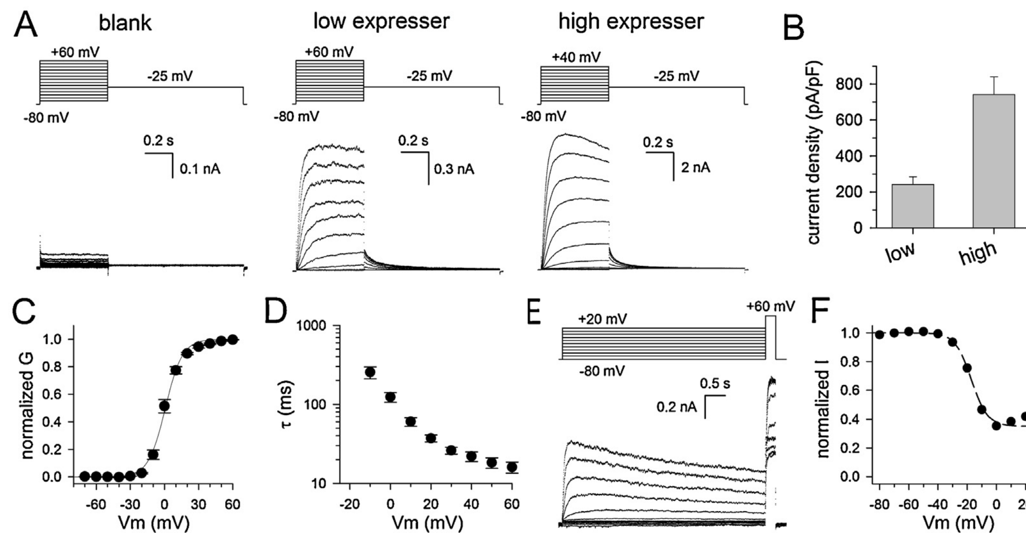

Fig. 1. Biophysical properties of the high and low Kv2.1 expressing cell line.
A, Representative ionic current recordings of blank *Ltk*⁻ cells (left) and two different *Ltk*⁻ cell lines that were stably transfected with Kv2.1 cDNA. Both cell lines express the channel differently yielding a low current phenotype (middle to low Kv2.1expresser) and a high current phenotype (right, high Kv2.1 expresser). Currents were elicited using the pulse protocol shown on top and zero current level is indicated by the horizontal bar at the start of the current recordings. B, Bar chart showing the average current density ±SEM for the low (left) and high (right) Kv2.1 expressing *Ltk*⁻ cell line. The current density was calculated by normalizing the steady-state current amplitude at +40 mV, obtained from recordings as shown in panel A, to the cell capacity, which is a measure for cell size. C, Conduction versus voltage GV curve of the Kv2.1 current in the low Kv2.1 expresser, which was obtained by plotting the normalized tail current amplitude from pulse protocols shown in panel A as a function of pre-pulse potential (data points are average ± SEM). Solid line represents the average fit with a Boltzmann equation. D, Plot shows the average voltage-dependent activation time constants ± SEM for the low Kv2.1 expresser (circles, $n = 8$). E, On top, the typical voltage protocol that was used to determine the voltage dependence of channel inactivation. After applying a 5 s depolarizing step the amount of channel inactivation was quantified by applying a +60 mV test potential. Below a representative ionic current recording of the low Kv2.1 expressing cell line. Note the gradual reduction in current upon prolonged depolarizing potentials, which reflects channel inactivation. F, Panel shows the voltage dependence of channel inactivation, which was obtained by plotting the normalized current during the +60 mV test potential from pulse protocols shown in panel E as a function of depolarizing pre-pulse. Note the Kv2.1 characteristic U-type inactivation profile with reduced inactivation at more depolarized potentials.

Several studies have highlighted the potential of ion channel specific immunoglobulins to treat a variety of diseases [11–13]. Immunoglobulins or antibody molecules are increasingly being developed for the treatment of tumors. They may represent a novel tool that can be exploited for designing by genetic engineering new Kv2.1 channel modulators. They also represent an interesting tool for immunostaining and biomarker identification. To develop antibody-based modulators of membrane proteins, we questioned whether immunizing dromedary with cells that express the mouse Kv2.1 channel protein is sufficient to induce an immune response and most importantly yields a serum with polyclonal antibodies that recognize and target the Kv2.1 channel. To address this question we developed *Ltk*⁻ cell lines that stably overexpress the Kv2.1 channel (Fig. 1). Dromedary serum contains, in addition to conventional heterotetrameric camelid IgG1, heavy chain immunoglobulins devoid of both light chains and conserved CHI domains (i.e. IgG2a and IgG3) [14]. Thus, the heavy chain-only antibodies recognize their cognate antigen by virtue of one single variable domain (instead of paired variable domains). This special antigen binding site prefers to associate with epitopes that are not antigenic for classical antibodies. This unique characteristic could be an advantage in differentially modulating the Kv2.1 channel via extracellular epitopes of the channel.

Herein, we have successfully developed a specific dromedary humoral immune response to Kv2.1 channels as demonstrated using an enzyme-linked immunosorbent assay on cells (Cell-ELISA). Furthermore, the serum of Day 51, which is the serum taken 51 days after onset of the immunization, was able to inhibit the Kv2.1 currents as evaluated by a patch-clamp assay. These results demonstrate that an immunization approach with *Ltk*⁻cells overexpressing the Kv2.1 channel yields polyclonal antibodies that are able to recognize and to modulate Kv2.1 channels through partial inhibition of the current amplitude without substantial change in the kinetics.

2. Materials and methods

2.1. Cell lines and cell culture

Ltk⁻cells were cultured in DMEM medium supplemented with 10% horse serum and 1% penicillin/streptomycin under a 5% CO_2 atmosphere at 37 °C. The *Ltk*⁻ cells were stably transfected with the mouse *Kv2.1* channel gene and were cultured in DMEM medium supplemented with 10% horse serum, 1% penicillin/streptomycin and 0.25 mg/ml G418. Both, low and high mouse Kv2.1 expressing *Ltk*⁻cell lines were constructed using a similar approach as described previously. Subconfluent cultures were incubated with 2 μM dexamethasone for 24 h to induce Kv2.1 channel expression and the harvested cells were used to immunize and for electrophysiological recordings [15].

2.2. Dromedary immunization

One, three years old and non-pubertal, male camel (*Camelus-dromedarius*) weighting 210 kg was provided by the local veterinary at Tantana, Sousse. After a period of quarantine, the dromedary was immunized with *Ltk*⁻cells expressing Kv2.1 channels, using an optimized protocol of immunization approved by the Comitéd'EthiqueBiomedicale, Institut Pasteur Tunis (Agreement number *CEBM 2015/12/I/LR11IPT08/V0*) and in accordance with the European Directive 2010/63/eu. Preparations of approximately 10^8 viable *Ltk*⁻cells expressing Kv2.1 channel were mixed with complete (first injection) or incomplete Freund's adjuvant (subsequent boosts) and injected, subcutaneously. The dromedary received successively four doses at days 0, 7, 21 and 51. Blood (5 ml) was collected before the first injection (i.e. Day 0) and during the course of the immunization, just before each injection. The sera were collected and stored at −80 °C until further use.

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