THE EFFECT OF CRYOPRESERVATION ON THE CURRENT RESPONSE OF CAV 3.1 TRANSFECTED HEK293 CELLS

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Abstract: The main disadvantage of long-term storage of living cells in liquid nitrogen is its relatively fast evaporation. For this reason, it is necessary to refill Dewar flask quite often. In our experiments, we used low temperature freezer (-80 °C) as more economical option to liquid nitrogen and we would like to show that there is no significant influence of long-term storage of mammalian cell-sat a low temperature. The effect of temperature was studied as the electrophysiological characteristic of the whole-cell Ca2+ current in HEK293 cells. The current responses were measured from cells stored for eight months at -80 °C and compared with previously published papers. The current traces and I-V curves showed that there are no changes in current response between cells frozen at a low temperature and previously published results. From our results can be concluded that the low temperature freezer is an adequate option for storage of mammalian cells for a couple of months.

Keywords: patch clamp, current response, cell freezing, HEK293

1. INTRODUCTION

Because mammalian cells loosing quality with increasing number of passages they cannot be passaged indefinitely. The best way to access high quality cells is to preserve them in a low passage number at frozen state in liquid nitrogen (LN2) for a long-term storage or in a low temperature freezer for a couple of months.. Main advantage of LN2 procedure is rapid cell/tissue freezing in the contact with LN2. However the liquid nitrogen also has some disadvantages. The main problem might be a fast LN2 evaporation from Dewar flask which required to be refilled quite often. This could be especially for small laboratories with limited number of experiments and accesses to liquid nitrogen a difficulty that must be somehow overcome. One approach is based on the cryopreservation of cells in low temperature freezers (e.g. -80 °C). These freezers are commonly available in many laboratories and are used for storage of chemicals and/or short-time preservation of cell tissues. In this paper the main goal is to measure the current response of Ca_V 3.1 transfected HEK293 cells as a result of long-term cryopreservation at a low temperature whether materials and methods.

2. MATERIALS AND METHODS

2.1. HEK293 CELLS AND CA_V 3.1 MEMBRANE CHANNEL

HEK293 cells originally come from human fetus aborted under Dutch law. Stable cell lines of HEK293 were generated in 1970s by Graham and van der Eb [1] at the University of Leiden, Holland. The name of cells is composed of cell type - *Human Embryotic Kidney*, and the number 293 comes from the number of experiments made by scientist. From 1970, HEK293 line expanded due

to its easy cultivation, rapid growth, easy transfection [2] and low calcium and sodium channel expression [3]. Characteristically, HEK293 membrane potential is about -40 mV [2].

As was mentioned above, HEK293 cells with stable Ca_V 3.1 transfection [4] were used in this work. Ca_V 3.1 belongs to T-type of membrane calcium channels activated at low voltage (LVA). Thus, LVA channels are characterized by its activations at low membrane potential (about +20 mV from holding potential – *HP*). Ca_V 3.1 channel consist of α1 with combination of other auxiliary β, α2-δ and γ subunits [5] and is characterized by very fast activation and relatively fast recovery from the inactivation. In addition, the presence of α2-δ subunits in Ca_V 3.1 channel may play role in perception of pain and heat [6]. This channel was also found in cardiac tissue and neurons [5]. The typical current response can be found on F 1b) or c).

2.2. CELL CULTURES

Cells were cultivated at 37°C and 5% CO_2 in Eagle's Minimum Essential Minimum (EMEM) supplemented with 10% FBS, 1% Penicilin/Streptomycin, 1% L-Glutamine and 1% G-418 as an antibiotic selector (all from Sigma-Aldrich). The cells were subcultured, following the standard protocol for adherent cells [7] once a week up to thirty passages as maximum.

2.3. PATCH CLAMP TECHNIQUE

The current responses were obtained by standard patch clamp technique which has been established by Hamill et al [8, 9] in 1980. This electrophysiological technique allows the user to study membrane currents (voltage clamp) and membrane potentials (current clamp) of many cells. Patch clamp operates in a several modes [9, 10]. The most used is probably whole-cell mode in which the cell membrane in ruptured by extra suction using a thin glass micropipette as a recording electrode. The pipette is filled with the solution similar to the intracellular fluid. Cells are during the measurement placed in bath solution which is similar to the extracellular fluid [10]. Both solutions can be supplemented with specific channel blockers [6]. Based on cell type, different stimulation protocols can be applied. For instance, for voltage-gated channels is used voltage-step protocol (Fig. 1a); stimulation protocol for optogenetic is used for light-gated channels integrated in cell membrane [11].

Experimental setup consists of Axopatch 200B amplifier and Digidata 1440 interface (Axon Instruments) and for current response analysis, Clampex and Clampfit 10.4 (Axon Instruments) were used. Patch clamp experiments were performed in whole-cell voltage – clamp configuration. Patch clamp pipettes prepared from borosilicate glass capillaries (1.5 mm external diameter; 0.38 mm wall thickness; World Precision Instruments) with the tip diameter between 1-3 μm and the resistance 2-3 MΩ.. Pipette solution with osmolality 317 $mosm \times kg^{-1}$ contained 130 mM CsCl, 10 mM TEA-Cl, 10 mM HEPES, 10 mM EGTA, 5 mM Na-ATP and 5 mM $MgCl_2$. The solution was titrated to pH 7.4 using CsOH. Bath solution with osmolality 322 $mosm \times kg^{-1}$ contained 140 mM NaCl, 10 mM HEPES, 10 mM D-Glucose, 5 mM EGTA, 2 mM $CaCl_2$ and 1 mM $MgCl_2$. The solution was titrated to pH 7.4 using NaOH (all from Sigma-Aldrich).

3. RESULTS AND DISCUSSION

For the statistical analysis, up to 15 experiments were performed with HEK293 cells in whole cell configuration and voltage-clamp (current recording) mode. Each experiment comprises at least ten cells. Based on the voltage stimulation protocol, the holding potential was kept at -100 mV and interrupted 16 - times by 10 mV steps (up to +50 mV) with 80 ms duration see example on Fig. 1a). The 3 seconds pause was applied between each period for sufficient channel recovery from inactivation.

The real experiment of recorded current from cell is shown in Fig. 1b. In comparison to previously published current response [6] (Fig. 1c) there are small differences in shape (time constants) of

sweeps and drifting at the end of sweeps. The maximal current is approximately 1300 pA. In conclusion the amplitude and the shape are very similar to previously published data [6] (Fig. 1e).

In case of I-V curves, there is no shift in maximum between our and published curves. The curve obtained from our experiment has maximum at -50 pA/pF between -10~-20 mV. Our curve also demonstrates a small shift (about 25 mV) of reversal potential which may be caused by low gigaseal quality or by current drift.

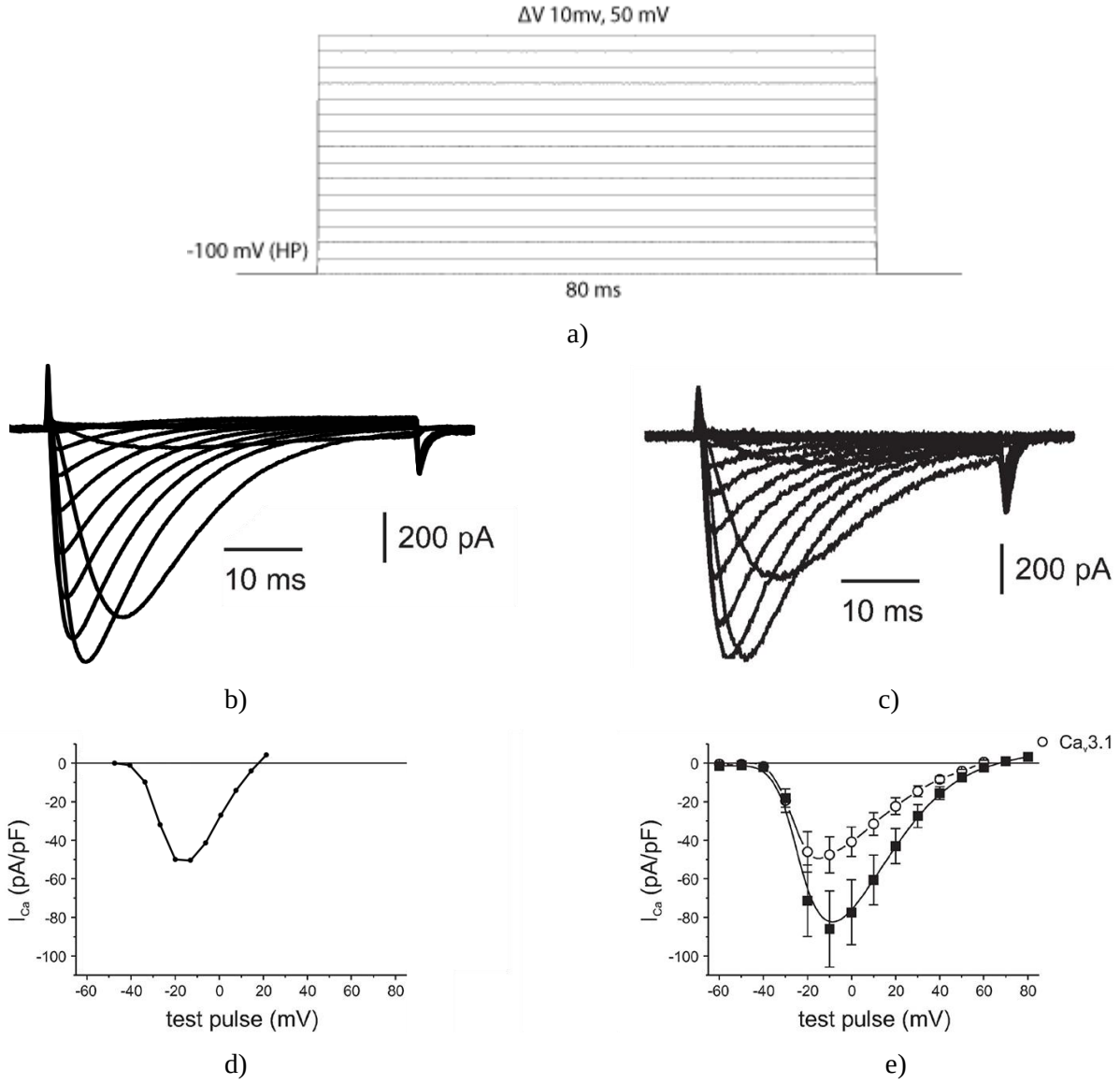

Fig. 1: a) CaV 3.1 voltage stimulation protocol; b) current response from our experiment ; c) previously published current response [6]; d) I-V curve from our experiment; e) previously published I-V curve [6].

4. CONCLUSION

In this paper, current responses and I-V curves from frozen HEK293 transfected by Ca_V 3.1 membrane channel is compared to previously published papers. No significant differences were found between results. From our findings can be concluded that the long-term storage of cell at low temperature storing can serve as an alternative to liquid nitrogen.

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