Lack of novel current observed in HEK293 cells expressing NALCN ion channel.

Jennifer Egan, Colleen S Peterson, W Mark Fry

Department of Biological Sciences, University of Manitoba

NSERC CRSNG

University of Manitoba

Background

The NALCN (sodium leak channel, non-selective) gene product is thought to form a voltage-independent, non-selective cation leak channel[1]. In some cells NALCN is thought to influence resting membrane potential of excitable cells and contribute to tonic depolarization facilitating spontaneous activity[2,3], whereas in others it is activated by G-protein-coupled receptors. Among these are muscarinic receptors[4] and receptors for neurotensin and substance P[5].

Furthermore, one group expressed NALCN in HEK293 cells and were able to generate a muscarinic current through NALCN[4].

We sought to investigate the properties of these ion currents stimulated by activation of M3R in NALCN transfected HEK293 cells.

Methods

Cell culture

HEK293 cells were grown in Dulbeco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), at 37 °C with 5% CO_2.

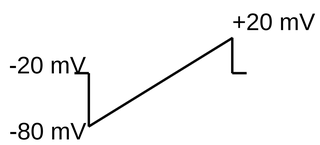
Cells were transfected using Lipofectamine 2000 with one of four treatments:

- No transfection
- eGFP only
- M3 muscarinic receptor (M3R) + eGFP
- NALCN, NALCN accessory proteins (UNC-80 and Src), and M3R + eGFP

Electrophysiology

Experiments were performed in whole cell voltage clamp at room temperature with constant perfusion of extracellular recording solution at a rate of ~1 mL/min.

Cells were held at -20 mV and current was recorded during depolarizing voltage ramps from -80mV to +20mV over 1000ms.



Statistical analysis

Cells exhibiting over 50% increase in current at 20 mV after drug application were considered responders. Current at 20 mV between groups before and after drug application were compared using two-way mixed-design ANOVA followed by Bonferroni post hoc tests. Reversal potentials were determined by plotting current against voltage at peak response, and compared using one-way ANOVA with Bonferroni post hoc tests.

Results

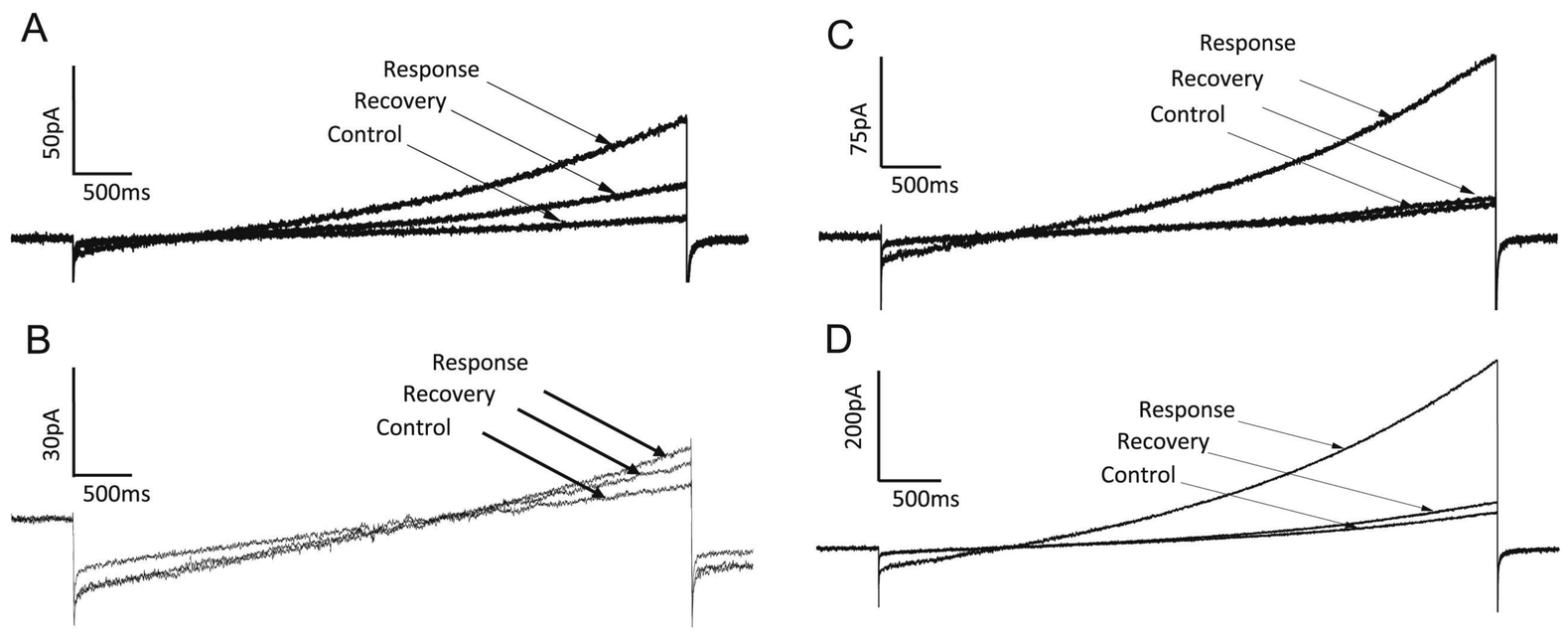


Figure 1. Representative currents elicited by voltage ramps (-80 mV to +20 mV over 1000 ms) from (A) untransfected cells, (B) eGFP cells (C) M3R cells, and (D) M3R+NALCN/Unc80/Src cells before application of muscarinic receptor agonist (100µM muscarine iodide or 100µM Oxotremorine M) for 1 minute. Control, response and recovery indicated by arrows.

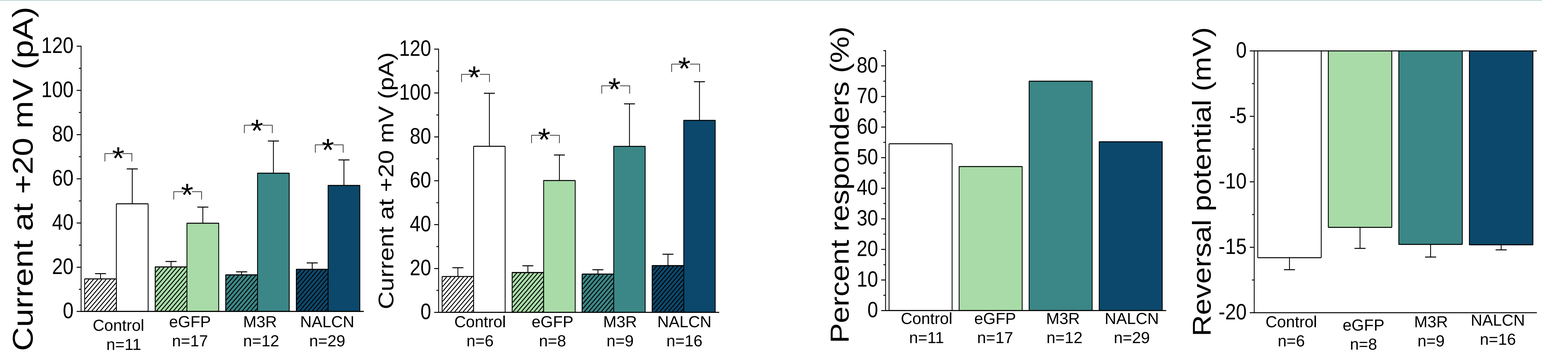


Figure 2. Currents observed in M3R and M3R+NALCN/Unc80/Src (NALCN) cells exhibited similar properties to currents observed in untransfected cells and eGFP transfected cells. A and B display The amplitude of the current at +20mV before and after application of muscarinic receptor agonist (muscarine iodide or Oxo-M) at 100µM for 1 minute. For all cells tested, (A), there was a significant main effect between the current amplitude before and after agonist .There were no significant between subject effects . For responsive cells only, there was a significant main effect between the current amplitude before and after agonist .There were no significant differences between subject effects.
(C) Percentage of untransfected, eGFP, M3R and NALCN cells considered responsive to muscarinic receptor agonist application were not significantly different (p=0.51, Chi-square test).
(D) Mean reversal potentials for currents elicited from responsive untransfected, eGFP, M3R and NALCN cells were not significantly different .

Conclusions

Our work supports previous work which suggests that previously recorded NALCN leak currents in HEK293 cells were the results of poor membrane-glass seals[6].
NALCN + UNC-80 + Src do not form a functional channel in HEK293 cells. Other accessory proteins may be necessary. Currently, cells which endogenously express NALCN such as primary neurons and MIN6 cells are the best available systems for the study of NALCN.

Literature

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