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Effect of the beta-1 subunit on electrophysiological properties of voltage-gated Na+ channels in MDA-MB-231 breast cancer cells

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Introduction: Voltage-gated Na^+ channels (VGSCs) are heteromeric protein complexes formed of a pore-forming α-subunit and one or more auxiliary β subunits. VGSCs increase invasion and migration in the triple-negative MDA-MB-231 breast cancer cell line. Overexpressing β1 in MDA-MB-231 cells (MDA-MB-231-β1-GFP) increases peak Na^+ current density, although the mechanism(s) are unclear(1). β subunits are proteolytically processed by secretases, releasing intracellular domains (ICDs), which may in turn transcriptionally upregulate α-subunits(2). In this study, we aimed to investigate the mechanism by which β1 increases Na^+ current in MDA-MB-231 cells.

Method: Whole-cell patch clamp recording was used to isolate and quantify VGSC currents in voltage clamp mode. Cells were perfused with either 1 μM TTX or PF-04856042, an $Na_V1.7$-specific blocker. To inhibit γ secretase, cells were treated with DAPT (0.5 μM), for 24 hours prior to recording.

Results: Application of 1 μM TTX significantly reduced the peak Na^+ current density in MDA-MB-231-β1-GFP cells from -14.3 ± 2.6 pA/pF to -9.6 ± 1.8 pA/pF (n=12, P<0.01) whilst having no significant effect in control MDA-MB-231-GFP cells. Treatment of MDA-MB-231-β1-GFP cells with DAPT significantly reduced the peak Na^+ current density from -19.5 ± 2.4 pA/pF to -4.3 ± 2.7 pA/pF (n=12, P<0.001) but did not significantly alter the current density in control MDA-MB-231-GFP cells. Perfusing MDA-MB-231-β1-GFP cells with PF-04856042 significantly reduced the peak Na^+ current density from -19.7 ± 2.6 pA/pF to -5.0 ± 0.6 pA/pF (n=10, P<0.001) but had no significant effect in control MDA-MB-231-GFP cells. $Na_V1.7$, which is up-regulated in lung cancer cells, displays a unique subthreshold current (3, 4). A depolarising ramp protocol revealed an inward current in MDA-MB-231-β1-GFP, but not MDA-MB-231-GFP cells, consistent with $Na_V1.7$ expression in the former. Perfusion with PF-04856042 significantly inhibited this current in MDA-MB-231-β1-GFP cells, but had no effect in control MDA-MB-231-GFP cells (n=10, P<0.0001).

Conclusion: Our data suggest that β1 up-regulates the expression of $Na_V1.7$ in MDA-MB-231 cells, likely via a mechanism that requires proteolytic release of the β1-ICD. Combining PF-04856042 application with ramp recording may represent a useful new approach for studying $Na_V1.7$ expression/activity in cancer cells.

References:

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