Novel modulation of Na_v channels in dorsal root ganglion neurons by veratridine

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The expression and activity of voltage-gated Na^+ channels (Na_vs) is a major determinant of neuronal excitability. While the modulation of Na_vs on the transcriptional level is relatively well studied, the mechanisms regulating Na_v cell surface levels remain largely unknown. One mechanism for the regulation of the epithelial Na^+ channels (ENaC) is by ubiquitination followed by endocytosis and proteasomal degradation. Internalization of ENaC is triggered by an elevation of intracellular [Na^+] which via a feedback mechanism leads to the activation of Nedd4 ubiquitin ligases and subsequent ubiquitination of ENaC (Dinudom *et al.,*, 1998). A rapid Na^+-induced Na_v internalization has also been reported to regulate Na_v cell surface levels in embryonic cortical neurons (Paillart *et al.*, 1996), however, it is also not known whether this mechanism is relevant in other neuronal cell types. This activation-induced internalization of Na_v channels is likely to reflect a protection mechanism by which neurons can respond to increased external stimuli by reducing their ability to generate action potentials. Such a mechanism may be of particular importance in dorsal root ganglion (DRG) neurons as significant alterations of the Na^+ current density and Na^+ subtype profile in the DRG contribute to the pathogenesis of pain states. In the current study, we investigated whether activation of Na_vs and elevation of intracellular [Na^+] in DRG neurons lead to an alteration of the Na^+ current density.

Treatment of cortical neurons with the neurotoxin veratridine activates Na_v channels by inhibiting channel inactivation and inducing persistent opening of the channels. This in turn leads to an increase in intracellular Na^+ that results in a reduction in the whole-cell Na^+ current density. To determine if veratridine had a similar effect on DRG neurons, we incubated primary cultures of DRG neurons with veratridine at 37°C for different periods of time after which Na^+ current density was measured using whole-cell patch clamping. The bath solution consisted of (in mM) NaCl (70), KCl (5), $CaCl_2$ (2), $MgCl_2$ (1.5), NMDG-Cl (50), Hepes (10), TEA-Cl (10) and $CdCl_2$ (10), whereas the pipette solution contained (in mM) CsF (130), CsCl (15), NaCl (5), Hepes (10) and EGTA (10). Veratridine reduced the Na^+ current density with a half-life of ~15 min and steady-state was reached after ~45 min, at which time only approximately 20 % of the original Na^+ current density remained. Next we investigated whether this effect was due to Na^+. The experiments were repeated in bath solutions where Na^+ was replaced with equimolar $NMDG^+$. Under these conditions, veratridine failed to reduce the Na^+ current density. Furthermore, the veratridine-mediated down-regulation in Na^+ current density was mimicked by elevating the Na^+ concentration in the pipette solution from 5 to 70 mM. This resulted in a significant reduction in Na^+ current density during whole-cell recording over a period of 20 min. To determine if the down-regulation of Na^+ current density in DRG neurons by veratridine was due to alterations in Na_v trafficking, DRGs were incubated with veratridine first for 3 min at 37°C to activate Na_vs and then at 4°C for 1 hour to inhibit trafficking. Under these conditions, veratridine had no effect on Na_v current density in the neurons, suggesting that the Na^+-induced reduction in Na^+ current density may involve trafficking of Na_v channels.

Taken together, these data suggest that elevation of intracellular [Na^+] leads to a decrease in Na^+ current density of DRG neurons, which may be due to increased Na_v endocytosis as reported for Na_vs in the embryonic brain and ENaC in the kidney. The cellular mechanisms behind this process remain to be elucidated and future studies will investigate the potential involvement of Nedd4 ubiquitin ligases.

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Paillart C, Boudier JL, Boudier JA, Rochat H, Couraud F, Dargent B. (1996) *Journal of Cell Biology*, **134:** 499-509