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Resistance of presynaptic CaV2.2 channels to voltage-dependent inactivation: Dynamic palmitoylation and voltage sensitivity

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Abstract

Presynaptic CaV2.2 (N type) calcium channels gate the influx of calcium ions to trigger transmitter release. We have previously demonstrated at the chick ciliary ganglion presynaptic calyx terminal that the bulk of these channels are highly resistant to voltage dependent inactivation [E.F. Stanley, G. Goping, Characterization of a calcium current in a vertebrate cholinergic presynaptic nerve terminal, J. Neurosci. 11 (1991) 985–993; E.F. Stanley, Syntaxin I modulation of presynaptic calcium channel inactivation revealed by botulinum toxin C1, Eur. J. Neurosci. 17 (2003) 1303–1305; E.F. Stanley, R.R. Mirotznik, Cleavage of syntaxin prevents G-protein regulation of presynaptic calcium channels, Nature (Lond.) 385 (1997) 340–343]. Recent studies have suggested that CaV2.2 can be rendered inactivation resistant when expressed with the palmitoylated β2A subunit and that this effect can be eliminated by tunicamycin, a general inhibitor of dynamic palmitoylation [J.H. Hurley, A.L. Cahill, K.P. Currie, A.P. Fox, The role of dynamic palmitoylation in Ca^{2+} channel inactivation, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 9293–9298]. We find that while tunicamycin treatment had no effect on CaV2.2 current in the inactivation-sensitive isolated chick dorsal root ganglion (DRG) neuron, it caused a 10 mV hyperpolarized shift in the profile of the inactivation-resistant presynaptic CaV2.2 population. This shift occurred without any effect on the voltage sensitivity of the inactivation process, as measured by a Boltzmann slope factor. Our findings suggest that dynamic palmitoylation contributes to the hyperpolarized steady inactivation profile of presynaptic CaV2.2. However, some other factor must also contribute since its inhibition does is not restore the inactivation profile to that of channels in the cell soma.

Keywords: Calcium channel; Voltage-dependent inactivation; Presynaptic; N-type; Transmitter release

1. Introduction

CaV2.2 voltage gated ion channels play a critical role in the gating of transmitter release from presynaptic terminals. Although somatic CaV2.2 are routinely used as experimental models for those at the presynaptic terminal these two populations exhibit significant functional differences, in particular with respect to voltage-dependent inactivation. A hyperpolarized voltage-dependent inactivation (VDI) range was one of the cardinal features used to distinguish calcium current through CaV2.2 from other high voltage activated calcium channel types [5,6], although inactivation-resistant CaV2.2 current was also recognized [1,7,8]. Thus, at neuronal somata and when expressed in non-neuronal cells CaV2.2 channels typically exhibit half inactivation voltages ($V_{50\%}$) in the range of −80 mV to −45 mV [9–11]. However, we originally noted that when recorded in native presynaptic terminals the bulk of the CaV2.2 calcium current is remarkably *resistant* to VDI [1,2,12,13]. Indeed, at least as far as can be ascertained from published current traces, this appears to be a common property for most, if not all, presynaptic calcium currents regardless of the channel type [14–19].

When examined in detail, the presynaptic CaV2.2 current was composed of two distinct Ca^{2+} current populations with respect to VDI: a small population with a hyperpolarized

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inactivation profile (∼25%; $V_{50\%}$ ∼−75 mV; VDI-sensitive) and a much larger population with a much more depolarized, and hence inactivation resistant, voltage profile (∼75%; $V_{50\%}$ ∼−13 mV; VDI-resistant) [2]. At the normal resting potential of ∼−65 mV virtually all of the former component must be in an inactive state and, hence, the latter, VDI-resistant must be responsible for transmitter release. Based on cleavage with Botulinum toxin C we have shown that intrinsic syntaxin 1 shifts the VDI-resistant inactivation profile $V_{50\%}$ approximately 8 mV in the hyperpolarizing direction. This effect was the same in polarity but far less in amplitude, with the effect of syntaxin 1 on the channel in cellular expression systems. Thus, syntaxin 1 inhibits CaV2.2 via a hyperpolarizing shift in VDI [10,20,21]. Since the syntaxin effect was minor and in the opposite direction it clearly cannot account for the marked resistance of the presynaptic CaV2.2 current to VDI and the underlying mechanism remains a mystery.

Recent reports suggest that steady-state inactivation in this channel requires a mobile I-II cytoplasmic loop [4,22]. This region of the Ca^{2+} channel also includes the beta subunit (CaVβ) binding site. Thus, CaV2.2 channels expressed with any of the CaVβ subunits other than CaVβ2a exhibit a hyperpolarized voltage-dependent inactivation. However, CaVβ2a greatly impedes inactivation due to an N terminal tail that is palmitoylated and, hence, anchored in the membrane [23–31]. A recent study noted that since palmitoylation is a dynamic process [32], it is possible to test for an association of the channel with CaVβ2a with the palmitoyltransferase inhibitor, tunicamycin [4]. Thus, tunicamycin liberates CaVβ2a from the membrane and the voltage-dependent inactivation properties of the CaV2.2 channel are shifted in the hyperpolarized direction towards that observed with the other, non-palmitoylated, CaVβ types [4]. Since both the rate of CaVβ2a palmitoylation turnover [26] and the rate of tunicamycin action [33] are rapid, this biochemical process can be examined using acute experimental procedures.

We have tested whether dynamic palmitoylation is involved in the resistance of presynaptic CaV2.2 to inactivation. Presynaptic CaV2.2 currents were recorded in chick ciliary ganglion calyx-type presynaptic terminals. Chick dorsal root ganglion (DRG) neurons, in which virtually all the calcium current is carried by CaV2.2 [34], were used as a somatic control.

2. Methods

2.1. Chick DRG neuron somata

E15-day chick embryos were decerebrated immediately after removal from the egg. Dorsal root ganglia were dissected from the lumbar region and incubated in modified Eagle's medium (MEM) with 0.588 mg ml^{-1} collagenase type IV (Worthington, Lakewood, NJ, USA), 11.8 mg ml^{-1} dispase (Boehringer Mannheim, Mannheim, Germany), 221 units ml^{-1} hyaluronidase (Worthington) and 0.110 mg ml^{-1} trypsin inhibitor type II (Sigma, St. Louis, MO, USA) for 2 h at 37 °C in 8% CO_2 and 92% air. The cells were washed, triturated and allowed to adhere to acetone-wiped glass coverslips for 90 min. The cells were rinsed twice more and maintained at 20 °C in 8% CO_2/92% air.

2.2. Chick ciliary ganglion calyx presynaptic terminal

The preparation of the chick ciliary ganglion presynaptic calyx synapse for the recording of presynaptic calcium currents has been described extensively [1,35–37]. Briefly, E15 day chick ciliary ganglia were dissociated by gentle trituration after treatment at 37 °C in an enzyme cocktail consisting of: collagenase-VI; hyaluronidase (Worthington); neutral dispase (Boeringer-Mannheim) together with trypsin inhibitor (Sigma), as Section 2.1). The ganglia were then triturated gently and allowed to adhere to coverslips at 37 °C before further experimentation.

Two of the advantages of this preparation as a presynaptic experimental model are that we can carry out reliable, if technically challenging, patch clamp recording under visual control and that the calcium channels are well characterized and virtually entirely CaV2.2 [1,12,35,38].

2.3. Whole cell patch-clamp recording

Currents were recorded using the whole-cell variant of the patch-clamp technique with 2.5–3.5 MΩ resistance fire-polished patch electrodes (thin wall, 1.5 mm outside diameter, World Precision Instruments). Recorded currents were amplified, and cell membrane capacitance was compensated electronically (Axopatch 200B; Axon Instruments, Foster City, CA). Voltage protocol generation and data acquisition are performed using pClamp V8 or V9 software. Currents were low-pass filtered at 10 kHz on-line and leak currents subtracted with a standard P/6 protocol using a positive-polarity leak subtraction pulse (following the test trace for pre-pulse inactivation protocols). Traces were digitally filtered at 2 kHz for analysis and display. All recordings were performed at room temperature. Whole-cell calcium currents were isolated using the following solutions. The external (bath) medium is composed of (in mM): NaCl 143, $CaCl_2$ 2, $MgCl_2$ 0.8, D-glucose 5, 4-aminopyridine 2, tetrodotoxin 0.001, HEPES-Na 10, tetraethylammonium-Cl 20, $Ni(ClO_4)_2$ 0.1; with an osmolarity of 300 mOsm and pH 7.4. The patch electrode intracellular solution was: Cs-gluconate 105, CsCl 30, EGTA-Cs 10, $MgCl_2$ 1, HEPES-Cs 10, tetraethylammonium-Cl 20, MgATP 2; osmolarity 310 mOsm, pH 7.4.

2.4. Drug treatments

10 mM tunicamycin (Sigma) was prepared in DMSO and stored at 4 °C. Cells were placed in MEM containing freshly diluted tunicamycin (10 μM, 0.1% DMSO) for 1 h at 37 °C.