CARDIAC CHANNELS IN CAVEOLIN-RICH MEMBRANE DOMAINS: REGULATION OF SINGLE SODIUM CURRENT AMPLITUDE

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Introduction

Neurohumoral regulation of the cardiac sodium channel (Nav1.5) via the stimulation of β-adrenergic receptors is of particular interest in light of its effect under conditions of stress and cardiac disease. Beta-adrenoceptor stimulation affects the Nav1.5 channel by at least two major parallel pathways. The classical signal transduction paradigm is dependent on the phosphorylation of ion channels by protein kinase A (PKA-dependent pathway). Phosphorylation of the Nav1.5 channel results in changes in the voltage-dependent availability, kinetics of current decay and the amplitude of the whole-cell current (Matsuda et al., 1992; Ono et al., 1993; Schreibmayer, 1999; Lu et al., 1999). Gsα also diverges to interact with downstream proteins (PKA-independent). The overall objective of our research is to understand the PKA-independent signaling pathway of the β-adrenergic enhancement of Nav1.5 channels in adult ventricular cardiomyocytes. The sodium channel increase is central to the increase in action potential upstroke velocity and thus the increase in conduction velocity in the heart. Recently, we made observation that sodium cardiac channels function can be modulated by caveolin-3 in ventricular cells (Yarbrough et al., 2002; Palygin et

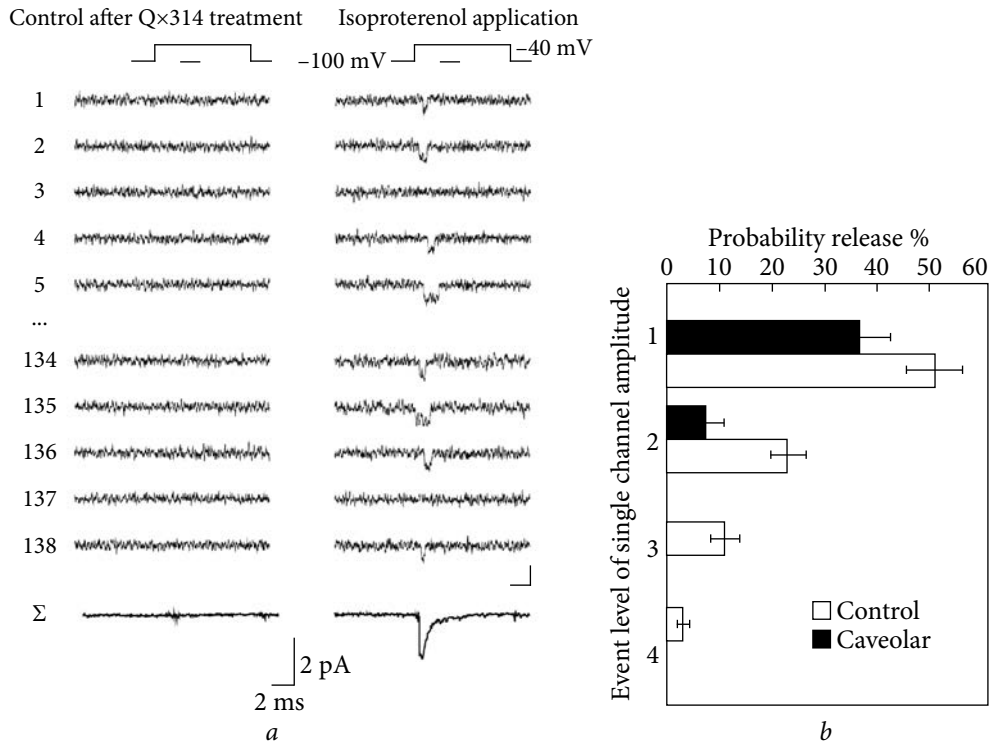

Fig. 1. The ISO-enhanced single sodium channel currents activity from ventricular cardiomyocytes caveolae invaginations. *a* — the absence of single channel activity after short depolarization pulse generating in the case of QX-314 application and blockade of the sodium conductance through the plasma membrane (left panel). Currents through a caveolar channels after β-receptor stimulation by 10 μM isoproterenol on cell incubated in QX-314 (right panel). Below, ensemble average of all recordings. *b* — probability release of single channel activity in regular normal single channel regording and in the case of isoproterenol induced caveolar Na channel activity after QX-314 treatment. Event levels correspond to quantal size of the single channel activity

al., 2008). Caveolin-3 is the signature protein marker that is found primarily in skeletal, smooth and cardiac muscle caveolae. Mutations of caveolin-3 were found to exhibit a persistent sodium current which presented characteristics of long QT-syndrome (LQTS9) and sudden infant death syndrome (SIDS, LQTS3-like) (Park et al., 2002; Hnasko and Lisanti, 2003; Cagliani et al., 2003; Vatta et al., 2006; Maguy et al., 2006). There are fundamental mechanisms of caveolae sodium channels function that are unknown. In this study, we demonstrate that increase of activity of Na+ channels in rat ventricular myocytes is directly regulated by Gsα-mediated PKA-independent pathway. And this direct regulation enhances INa through an increase in the number of functional NaV1.5 channels in the cell membrane. We show that these channels specifically localize to caveolar membranes.

The Nav1.5 channels properties in caveolin rich membrane domains

To consider the functional nature of the Na^+ current in caveolae, we isolated non-caveolae Na^+ channels found in the membrane surface from those found in caveolae vesicles. We took advantage of the compound QX-314, a quaternary derivative of the local anesthetic lidocaine (Alpert et al., 1989b). QX-314 is a permanently charged membrane impermeant blocker of Na+ channels, which has been shown to have both an intracellular and extracellular binding sites. Whereas car-

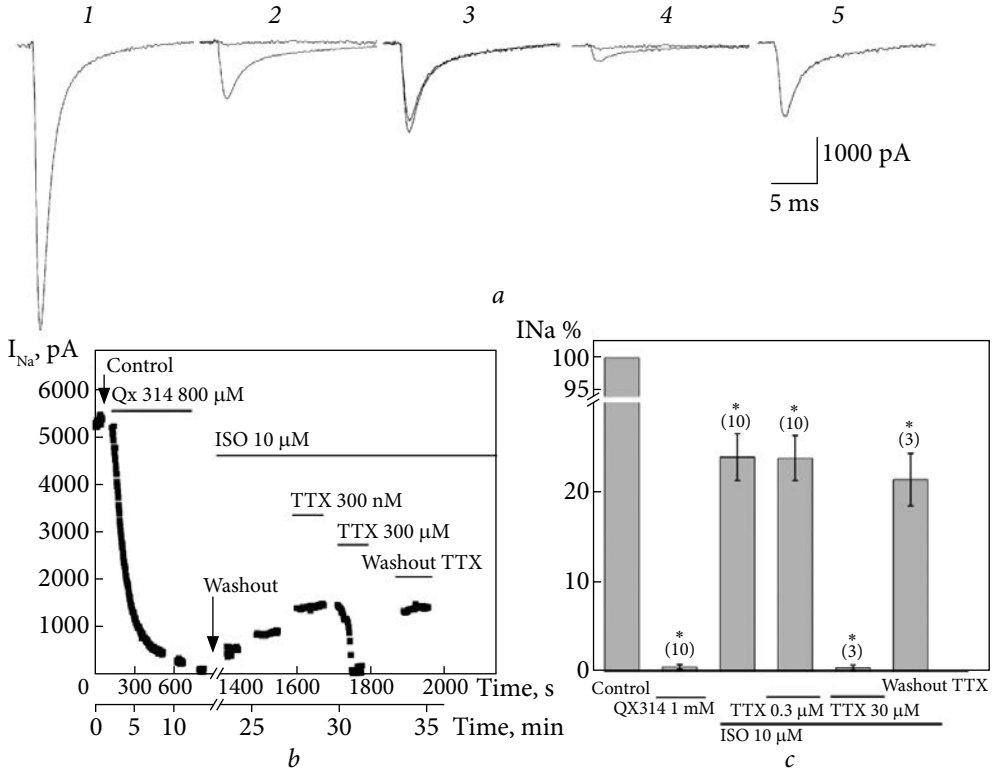

Fig. 2. The ISO-enhanced INa properties in the presence of TTX after QX-314 blockade of Na^+ conductance: *a* — sodium currents were elicited by stepping the membrane potential to –30 mV from a holding of –100 mV: (*1*) control, (*2*) complete block in the presence of QX-314, (*3*) application of 10 μM ISO and 0.3 μM TTX, (*4*) essential block by 30 μM TTX, (5) washout of TTX; *b* — the time course of experiment shown in *a*. The addition of ISO induces an increase in the voltage-gated INa after complete block and washout with QX-314. Application of a low concentration of TTX (0.3 μM) does not affect a beta-adrenergetic receptor induced INa. Application of a high concentration of TTX (30 μM) reversibly blocks ISO induced INa; *c* — relative percent change of INa in response to ISO and TTX under the conditions outlined in parts *a* and *b*. The number of observations is indicated in brackets above columns; asterisks show cases of significant differences from the control values; asterisks indicate cases of statistically significant values ($P < 0.05$)

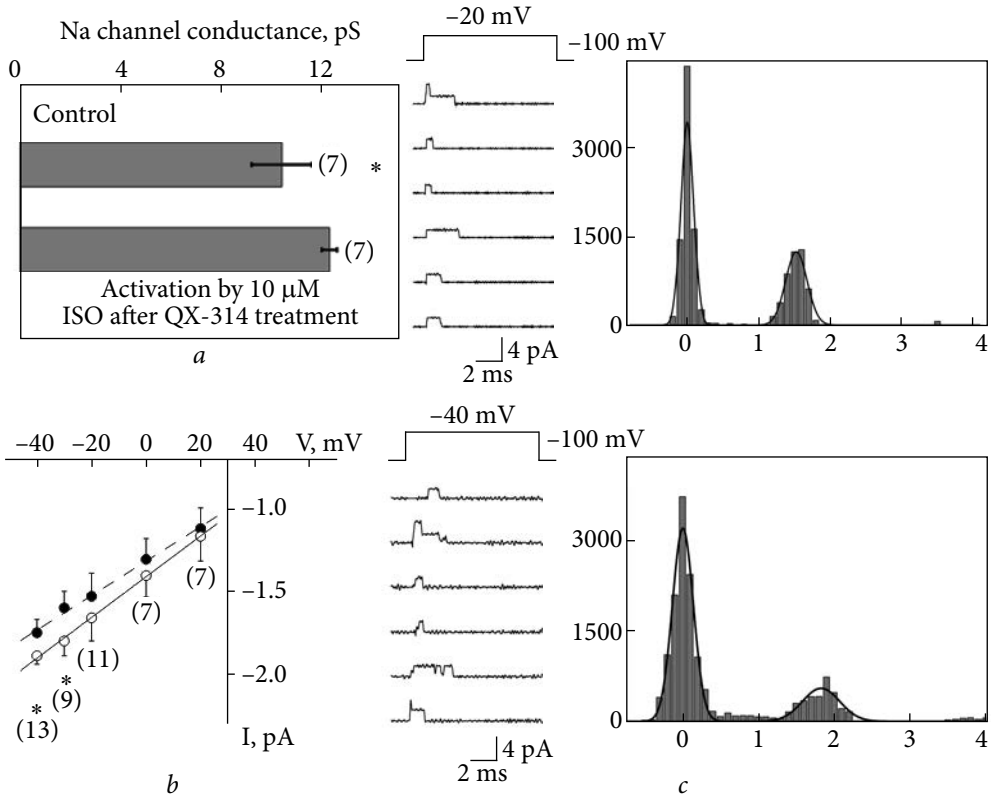

Fig. 3. Caveolae single channel activity in adult cardiac myocytes: *a* — cardiac myocytes single channel conductance in case of membrane surface channels 10.4 pS (n = 7) (control bar) and in case of caveolar channel activated by 10mkM ISO after 1mM QX314 blockade of surface sodium channels 12.3 pS (n = 7). Statistical difference was tested by analysis of variance with $P < 0.05$ (asterisks); *b* — comparison of the single Na channel IV relation in control and in case of isoproterenol induced caveolar Na channel after QX-314 treatment (conductance equal to 10 and 12.5 pS, correspondingly). Each point represents theamplitude sum of the first quantum level for averaged number of cells (in brackets) calculated in Single Channel Event Detection Module in Clampfit 9.0 (Axon Instruments). The reversion potential close to calculated with Boltzmann equation for 140 mM Na in pipette and 10-12 mM Na inside cell. Statistical difference was tested by analysis of variance with $P < 0.05$ (asterisks); *c* — all points amplitude histogram like sum of two normal distributions of single channel caveolae activity for two different voltage level steps (examples of single channel activity are indicated in inset)

diac Na^+ channels can be inhibited with QX-314 application from outside, neuronal Na^+ channels are inhibited only from the inside binding site (Alpert et al., 1989a; Qu et al., 1995). The usefulness of this compound is its very long dissociation kinetics (t½ of recovery > 1.5 h).

Aliquots of enzymatically isolated adult rat ventricular myocytes were electrically paced using field stimulation at a rate of 1 Hz before starting single chan-

nels experiments. Cell contraction was used to adjust the stimulation parameters (10 msec duration) and the constant current intensity was increased by 20%. In the presence of 1 mM QX-314, cells were paced for at least 10 min then washed for 5 min with QX-314-free solutions. This is a critical step since QX-314 in the solution may inhibit with time any Na^+ channels when the caveolae necks open with stimulation by ISO or Gsα. Initially, cell-attached patch clamp methods will be used to record single channel current, Fig. 1.

The data in Fig. 1 show a cell after field stimulation in 1 mM QX-314 for 10 min and washed with solution not containing QX-314 before making a pipette-membrane seal. The left panel shows a cell-attached patch stimulated with the voltage protocol shown at the top and filtered at 5 kHz (at -3 dB). The pulse starts at the onset of the voltage protocol and ends 10 msec later. The 10/138 representative control traces showed no openings in all 138 traces. This is consistent with our previous data where QX-314 blocked all of the surface Na+ channels irreversibly. The right panel shows the same membrane patch using the identical voltage protocol and stimulated with 10 μM ISO in the bath solution. The 10 selected current traces show a single channel. None of the sweeps showed multiple openings. Empty sweeps constituted about 55/138 sweeps (40%). The probability of release of the caveolae single channel currents is lower than in case of regular membrane recordings (Fig. 1, *b*).

That is due to much lower channel density in caveolae membranes. The whole-cell voltage-clamp measurements were conducted to evaluate the presence of different isoforms of voltage-gated Na^+ channels. Our data show that separate by QX-314 isoproterenol induced sodium current in the presence of PKA sensitive only to high concentration of TTX (Fig. 2). As a result, these single channel experiments (n = 20) allow usto analyze clear caveolar sodium current (Fig. 3). This method gives valid information about caveolar sodium channels quantity, conductivity, and other properties.

Cardiac sodium current regulation via caveolin rich membrane domains

Caveolae have function as pre-assembled signaling complexes through compartmentalization of signaling molecules that interact with caveolin proteins or liquid-ordered liquid caveolar lipids. In the heart, a variety of signaling molecules co-fractionated with caveolae, and there residence in caveolae, or movement out of caveolae, is important for their function. Multiple studies have shown that Cav-3 expression is dramatically decreased in different models of cardiac hypertrophy. This study provides support for our model that a Gsα-cav-3 interaction leads to the opening of caveolae and to an augmentation of the cardiac INa, due to an increase in the number of active sodium channels. The unknown switch that opens the neck of the caveolae is the key determinant of the caveolar signaling model. In closed caveolae, channels and receptors that localize to the caveolar membranes are essentially locked and nonfunctional. This can be due to the elec-

trical discontinuity of the caveolar space with the extracellular space, or the inability of the ligand to enter the caveolae to stimulate its receptor.

Thus, we show that a subpopulation of NaV1.5 channels that is localized in caveolae of rat ventricle myocytes is part of a signaling complex directly regulated by beta-adrenergic stimulation. This regulatory signaling pathway is PKA-independent and depends upon the interaction of the Gsα protein with caveolin-3 scaffolding protein. These findings demonstrate that subcellular localization of NaV1.5 channels to caveolae macromolecular signaling plays a specific functional role in the direct Gsα-mediated increase in rat cardiac ventricular sodium current.

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