Targeted disruption of the voltage-dependent calcium channel α2/δ-1-subunit

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1Institute of Molecular Pharmacology and Biophysics, Department of Surgery, and 2Department of Pharmacology and Cell Biophysics, University of Cincinnati, College of Medicine, Cincinnati, Ohio; and 3Division of Analgesics, Drug Discovery, Johnson & Johnson Pharmaceutical Research and Development, Spring House, Pennsylvania

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Fuller-Bicer GA, Varadi G, Koch SE, Ishii M, Bodi I, Kadeer N, Muth JN, Mikala G, Petrashevskaya NN, Jordan MA, Zhang S, Qin N, Flores CM, Isaacsohn I, Varadi M, Mori Y, Jones WK, Schwartz A. Targeted disruption of the voltage-dependent calcium channel α2/δ-1-subunit. Am J Physiol Heart Circ Physiol 297:H117–H124, 2009. First published May 8, 2009; doi:10.1152/ajpheart.00122.2009.—Cardiac L-type voltage-dependent Ca2+ channels are heteromultimeric polypeptide complexes of α1-, α2/δ-, and β-subunits. The α2/δ-1-subunit possesses a stereoselective, high-affinity binding site for gabapentin (GBP) (1-aminomethylcyclohexane acetic acid; Neurontin), widely used to treat epilepsy, postherpetic neuralgic pain, and sleep disorders (8, 13). Of the remaining α2/δ-subunits, only α2/δ-2 has also shown a propensity for GBP binding, but the affinity is much lower than α2/δ-1 (18, 24).

It is clear that the α2/δ-2-subunits of VDCCs are associated with some disease states. A spontaneous mutation in the α2/δ-2 murine gene resulted in the Ducky mouse, demonstrating clinical signs of epilepsy (1, 3). Similar results to those obtained for the Ducky mouse were found in an α2/δ-2 knockout mouse (17). These results include ataxic gait, seizures, signs of cerebellar degeneration, and premature death. There were some differences noted in the pathology of the cerebellar region for these two mice. There were no pathological abnormalities noted in the hearts of the α2/δ-2 null mice. In conscious mice, there was a trend toward bradycardia observed during ECG and transthoracic echo. However, while under the influence of isoflurane, the null mice showed a significantly less-pronounced decrease in heart rate. A mutation in the α2/δ-4 gene introduced a premature stop codon that truncated one third of the protein and led to a cone-rod dysfunction in the visual system of mice (35). In addition, a mutation of the α2/δ gene (unc-36) in Caenorhabditis elegans has been identified, manifesting in a lack of adaptation (desensitization) to dopamine and serotonin, a phenotype indistinguishable from the unc-2 phenotype that is caused by lack of the α1-subunit (25).

CARDIAC L-type voltage-dependent Ca2+ channels (L-VDCCs) are heteromultimeric polypeptide complexes of α1-, α2/δ-, and β-subunits. The α1-subunit is auto-regulatory and harbors the channel pore, gating machinery, and modulatory drug binding sites (30). The accessory subunits (α2/δ and β) affect channel kinetics and are involved in the trafficking and inclusion of the α1-subunit into the membrane. The α2/2-subunit is closely associated with an extracellular loop of the α1-subunit (15) and linked to a small protein called δ (2, 9). Both the α2 and δ are encoded by the same gene, separated by proteolytic cleavage, and extracellularly linked through a disulfide bridge (9). Currently, four α2/δ-2-subunits, each encoded by separate genes, have been identified (4). The α2/δ-1, originally cloned from skeletal muscle (10), is ubiquitously distributed (18), with high levels of protein expression in brain, heart, skeletal, and smooth muscle (13). It possesses a stereoselective, high-affinity binding site for gabapentin (GBP) (1-aminomethylcyclohexane acetic acid; Neurontin), widely used to treat epilepsy, postherpetic neuralgic pain, and sleep disorders (8, 13). Of the remaining α2/δ-subunits, only α2/δ-2 has also shown a propensity for GBP binding, but the affinity is much lower than α2/δ-1 (18, 24).

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Localization analysis of a gene encoding the polymorphous α2/δ-1-subunit to chromosome 7q provided some evidence of the segregation of flanking markers in families susceptible to malignant hyperthermia (16). The gene was localized to chromosome 5 in the mouse in an earlier study (7). In addition, the α2/δ-1-subunit has been shown to be upregulated in response to spinal injury and neuropathic pain (20, 36, 37). However, the effect of a knockout of the α2/δ-1-subunit in an in vivo model has not been accomplished.

We have developed a viable conventional α2/δ-1 knockout mouse using a construct targeting exon 2 of α2/δ-1. As we expected, the α2/δ-1-subunit knockout mice display a lack of the high-affinity GBP binding site and altered L-type Ca2+ current in cardiomyocytes, without causing a significant change in the expression of other Ca2+ channel subunits.}

METHODS

The investigation conforms to the Guide for the Care and Use of Laboratory Animals, published by The National Institutes of Health.
The animals in the present study were handled according to approved protocols and animal care regulations at the University of Cincinnati. With the exception of the mice used for genotyping, the animals used for experimentation, including binding, Western blot analysis, histopathology, ex vivo analysis, and electrophysiology were all between 2 and 8 mo of age.

**Generation of the αδ-1 knockout mouse.** A 12-Kb genomic fragment containing exon 2 of the αδ-1 gene encoding residues I33-D59 [82 base pairs (bp)] was isolated and sequenced from a lambda mouse genomic DNA library (129 SVJ, Stratagene; La Jolla, CA). Mutations were introduced into exon 2 to include XhoI and NofI sites. The XhoI site was inserted using the nucleotides from L44 and V45 and the NofI site used L47, A48, and K49. Similarly, a NcoI site was introduced into the long arm (5′; 4.0 Kb) and an NdeI site into the short arm (3′; 2.3 Kb). The fragment between sites NcoI and NdeI was placed in the vector thymidine kinase gene elegans (pMCTK), and the neomysin resistance gene was introduced into the XhoI-NofI of exon 2. Figure 1A represents a schematic of the gene-targeting construct. This construct was electroporated into murine embryonic stem cells. After positive (G-418) and negative selection (gancyclovir), genomic DNA was isolated from 288 embryonic stem cell lines. These cell lines were screened by PCR to identify the cell lines with an integration of the full-length targeting region of the knockout construct. Following the testing of 10 of the positive cell lines, one cell line was found in which site-specific targeting occurred. This positive line was microinjected into C57Bl/6 blastocysts and implanted into F1 (C57Bl/
6X129J) pseudopregnant females. Two separate injections into blastocysts yielded 12 pups. From these two litters, seven chimeric animals were identified (5 of them at the level of 100% chimerism and 2 at 80% chimerism). The 100% chimeras were bred with Black Swiss females. The resulting offspring were genotyped via PCR to determine which male chimeras had gametes capable of passing the mutant allele to progeny. Screening of pups using PCR genotyping confirmed heterozygous (+/−) and null (−/−) animals.

PCR genotyping for wild-type, +/+, and −/− αβδ-1 murine pups. DNA was isolated from tail prep of 3-week-old murine pups. The DNA was then added to a cocktail containing Taq Polymerase (NEB), Taq buffer, dNTPs and three primers: primer A (5′-CATGGGTGTTGACAAAGATGCAAG-3′), primer B (5′-CTGCAAGCTATTGAGGCAGC-3′), and primer C (5′-ATTCTCAAGACTGTAGGAGC-3′). Using the first nucleotide of exon 2 as the initial bp, the primers were located as follows: primer A begins at 9, primer B begins at 1,448, and primer C begins at 2,065 (Fig. 1D). The PCR conditions were 35 cycles of 62°C annealing for 1 min, 72°C extension for 1 min, and 94°C denaturing for 1 min. PCR products were run on a 2.5% agarose gel. The expected bands were 346 bp for the wild-type (WT) and 635 bp for the −/− (Fig. 1E). The heterozygous mice had both bands.

Determination of the targeting construct integration and knockout of the αβδ-1 subunit. To confirm that the targeting construct integrated at the homologous genomic site (i.e., exon 2 of the δ1 gene), we attempted Southern blot analysis using probes from flanking regions of the construct. However, four independent attempts at this failed, due to the highly repetitive nature of the DNA in the intronic sequences flanking exon 2. We then took advantage of the fact that 15 bp of sequence were deleted from the second exon (Fig. 1B). Small line indicating P2) during construction of the targeting construct is integrated elsewhere in the murine genome, both targeting construct successfully replaced exon 2 of the δ1 gene. Using the

**GBP binding.** The [3H]GBP (American Radiolabeled Chemicals, St. Louis, MO) binding assay was performed as described by Gee et al. (12). Membranes (20 μg/mg) of mouse tissues were incubated with [3H]GBP at room temperature for 60 min. A separation of free ligand was effected by filtration through 0.3% polyethyleneimine-soaked GF/B filters. The filters were washed with 3 × 4 ml of 10 mM HEPES (pH 7.4). Radioactivity retained by the filters was determined by scintillation counting. Nonspecific binding was defined in the presence of 100 μM unlabeled GBP. Western blots for the Ca2+ channel subunits. Total protein, isolated as described previously (14), was separated on SDS-PAGE gel, transferred to nitrocellulose, and analyzed with the following primary antibodies: voltage-dependent L-type Ca2+ channel (Ca1.2) (ACC-003, 1:1,100, Alomone), calsequestrin (PAL-913, 1:2,500, ABO), β2a [1:2,500; generous gift from M. M. Hosey (6)], αβδ-1 (D219, 1:1,000; Sigma), αβδ-2 (AC-REIYKDRNRLFEQVEQNPC-amide, 1:500; custom-made antibody by Johnson & Johnson, not published), αβδ-3 [1:200; custom-made antibody by Johnson & Johnson (24)], αβδ-4 [1:100; custom-made antibody by Johnson & Johnson (24)], Ca.2.1 (C1353, 1:200; Sigma), Ca.2.2 (ABS154, 1:100; Millipore), B3 (C1978, 1:500; Sigma), and the protein loading normalized to GAPDH (4300, 1:4,000; Ambion).

**Histopathology.** Following anesthesia with ketamine (100 mg/kg)-xylazine (10 mg/kg), body weight (BW) measurements were taken. Hearts were removed via bilateral thoracotomy, weighed, flushed with PBS (pH 7.4), fixed in 10% buffered formalin, and processed for histopathology following routine methods.

**Ex vivo analyses, working heart.** Control and αβδ-1 (−/−) age-matched mice of either sex were anesthetized with ketamine (100 mg/kg)-xylazine (10 mg/kg) and injected with 1.5 IU of heparin intraperitoneally to prevent intracardiac blood coagulation. Isolated hearts were placed in the working heart mode as previously reported (27). Positive and negative first derivatives of intraventricular pressure (dP/dr), duration of contraction (time to peak pressure), and relaxation (time to half relaxation) were calculated. Data are presented as means ± SD. Statistical analysis was carried out using Student’s t-test for unpaired observations. Values of P < 0.05 were regarded as statistically significant.

**Isolation of cardiomyocytes.** Single ventricular myocytes were enzymatically dissociated from isolated hearts of WT and αβδ-1 (−/−) age-matched mice of either sex, as previously reported (40), with modifications. In brief, the heart was rapidly excised and placed in Tyrode solution containing (in mM) 120 NaCl, 5.4 KCl, 1.2 NaH2PO4, 5.6 glucose, 20 NaHCO3, 1.6 MgCl2, 10 2,3-butanedione monoxime, and 5 taurine, infused with 95% O2-5% CO2. All solutions were filtered and equilibrated with 95% O2-5% CO2 for at least 20 min before use. The heart was perfused in retrograde mode for 4 to 5 min, followed by perfusion with buffer containing 1 mg/ml collagenase Type II (Worthington) at 37°C. After 2 min of enzyme perfusion, 50 μM CaCl2 was added to the solution. After ~5 min, when the heart was demonstrating characteristics of global cardiac death, the enzyme was recirculated. The heart was perfused for an additional 8–12 min or until flow rate surpassed preenzyme flow rate. After perfusion, the ventricles were separated from the atria and minced. Only Ca2+ tolerant cells with clear cross striations and without spontaneous contractions or significant granulation were selected for experimental studies.

**Electrophysiological measurements.** The electrophysiological studies were performed as previously reported (22). The following protocol was used to obtain steady-state inactivation parameters of L-type Ca2+ current (ICa). From a holding potential of ~80 mV, a series of 1-s prepulses (from ~80 to +50 mV in 10-mV increments) were applied, followed by a 6-ms repolarization to the holding potential and a subsequent test depolarization to +10 (WT) or +20 mV (αβδ-1 (−/−)). Peak currents at the test pulse were normalized to the peak current amplitude elicited after the −80-mV prepulse and plotted as a function of the prepulse voltage. All recordings were made after 3 min of membrane rupture at room temperature (22–24°C). These experiments were carried out in 1.8 mM Ca2+, using Ca2+ as the charge carrier.

**Data analysis.** The individual activation data [current-voltage (I-V) curves] were fitted to standard Boltzmann equation in the form ICa = Gmax (Vm − Vrev)/[1 + exp((Vm − Vrev)/k)], where Gmax is the maximal conductance, Vrev is the membrane voltage, Vrev is the ICa reversal potential, Vrev is the half-activation potential, and k is the slope factor. The obtained parameters of Gmax and Vrev were then used to calculate fractional conductance at each Vm, G/Gmax, using the equation: G/Gmax = ICa/Gmax (Vm − Vrev)/[1 + exp(−(Vm − Vrev)/k)], where G is the total macroscopic conductance at Vm. The G-V curves were plotted with the values obtained from the fit of the I-V curves, using the following form of Boltzmann equation: G/Gmax = 1/[1 + exp((Vm − V0.5)/k)].

The time course of decay of ICa was fitted to a two-exponential equation by Chebyshev method on Clampfit version 6.03 (Axon Instruments). The fit interval started at 60 ms and ended at 440 ms.
For the steady-state inactivation curves, the normalized amplitude of the inward Ca^{2+} current evoked by the test pulse (+10 or +20 mV) is plotted as a function of prepulse voltage.

RESULTS

Confirmation of the integration of the targeting construct and knockout of the subunit. Tail DNA isolated from homozygous mice (Fig. 1C, lanes 1–4) produced only the 459-bp PCR product, whereas the WT DNA produced only the 83-bp product (Fig. 1C, lanes 9–12). PCR from heterozygous mouse DNA (Fig. 1C, lanes 5–8) produced both bands. We performed a PCR control that consisted of a 1:1 mixture of homozygous and WT tail DNA. PCR of this DNA resulted in both PCR products, confirming that the homozygous DNA does not inhibit the production of the 83-bp product. These results demonstrate that the α2δ-1 knockout gene-targeting event occurred precisely as predicted and indeed does replace the normal endogenous allele. The genomic-targeting PCR confirmed the results of the PCR genotyping for WT, +/-, and --/-- mice.

Loss of the high-affinity GBP binding site. The VDCC α2δ-1-subunit possesses a high-affinity binding site for GBP.

Table 1. Comparison of [3H]GBP binding sites in different tissues from α2δ-1 (−/−) mice,WT, wild-type. *Value is out of detectable range.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Bmax, fmol/mg</th>
<th>Kd, nM</th>
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<tbody>
<tr>
<td>Brain</td>
<td></td>
<td></td>
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<tr>
<td>WT</td>
<td>3,359 ± 29</td>
<td>25 ± 0.5</td>
</tr>
<tr>
<td>+/-</td>
<td>2,757 ± 390</td>
<td>36 ± 8.9</td>
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<tr>
<td>--/--</td>
<td>570 ± 81</td>
<td>25 ± 5</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>3,343 ± 961</td>
<td>21 ± 3</td>
</tr>
<tr>
<td>+/-</td>
<td>2,268 ± 370</td>
<td>20 ± 4</td>
</tr>
<tr>
<td>--/--</td>
<td>145 ± 18*</td>
<td>11 ± 4</td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>713 ± 227*</td>
<td>27 ± 12</td>
</tr>
<tr>
<td>+/-</td>
<td>423 ± 202*</td>
<td>27 ± 18</td>
</tr>
<tr>
<td>--/--</td>
<td>201 ± 193*</td>
<td>20 ± 7</td>
</tr>
</tbody>
</table>

Values are means ± SD. GBP, gabapentin; Bmax, binding maximum; Kd, dissociation equilibrium constant; WT, wild-type. *Value is out of detectable range.

To determine the differences in protein expression in α2δ-1 (−/−) mice, a GBP radioligand binding experiment was performed with skeletal muscle, brain, and heart tissue. As shown in Fig. 2 and Table 1, the α2δ-1 (−/−) animals displayed no measurable or very low levels of [3H]GBP binding in skeletal muscle and brain. The density of GBP binding in the α2δ-1 (+/−) animals was also reduced compared with WT in either brain or skeletal muscle. Though the α2δ-1 binding site in WT mouse was not detectable in any of the tissues, the binding density was reduced by at least 50% in either brain or skeletal muscle.

Fig. 3. Expression of Ca^{2+} channels in α2δ-1-deficient mice. A: Western blot analyses of Ca^{2+} channel isoform voltage-dependent L-type Ca^{2+} channel (Cav1.2) and β2a-subunits in heart tissue normalized to calsequestrin. B: Western blot analyses of α2δ-isoforms (1, 2, 3, and 4), Cav2.1, Cav2.2, and β3-subunit normalized to GAPDH in brain, heart, and skeletal muscle (SM). The numbers reported as molecular mass are in kilodaltons.
heart tissue was very low (713 fmol/mg), approximately fivefold lower than in WT skeletal muscle and brain tissues, the deletion of the α2δ-1 subunit further reduced the GBP binding sites in heart.

Western blot analyses of the Ca\(^{2+}\) channel subunits. The murine cardiac L-type VDCC complex is composed of α1,2 (α1C), α2δ-1δ-1, and β2-subunits. To determine whether an ablation of α2δ-1 expression altered the expression of other subunits in the complex, we first performed Western blot analyses of α1,2 and β2a-subunit protein levels on total protein from heart tissue from WT and −/− mice. No significant changes were observed (Fig. 3A). Since four α2δ-isoforms have been identified to date, we wanted to evaluate whether the deletion of the α2δ-1 isoform may result in a compensatory increase in expression of the other isoforms. In addition, since it is well known that the α2δ-1 subunit also associates with other Ca\(^{2+}\) channel pore-forming α1-subunits, such as α1a, α1c, α1d, and α1f, to form P/Q-, N-, and R-type Ca\(^{2+}\) channels, respectively, the expression of these subunits was also investigated. To quantify the impact on expression of these proteins, Western blot analyses of total protein, obtained from heart, brain, and skeletal muscle from WT, +/+, and −/− α2δ-1 mice, were performed for each Ca\(^{2+}\) channel isoform. Consistent with the GBP binding data, α2δ-1 protein expression was not detected at all in the α2δ-1 (−/−) mouse heart, brain, or skeletal muscle. In contrast, no significant changes in protein expression were observed for any of the other Ca\(^{2+}\) channel subunit isoforms tested (Fig. 3B).

Clinical characteristics and morphology. The α2δ-1 (−/−) animals clinically appear normal, although males display a tendency for bladder dilatation. Grossly, the α2δ-1 (−/−) hearts appear normal with no obvious cardiac hypertrophy or dilatation (Fig. 4). At 8 mo, the average heart weight (HW) and BW were not significantly different between the two groups; α2δ-1 (−/−) HW was 172.5 ± 18.9 mg, and BW was 29.3 ± 2.7 g (n = 4) compared with the WT HW of 154.3 ± 12.9 mg and BW of 28.8 ± 1.8 g (n = 7), respectively. HW-to-BW ratios also were not significantly different between α2δ-1 (−/−) and WT (5.9 ± 0.42 and 5.37 ± 0.28 mg/g, respectively). The majority of the animals were female. When analyzed by sex, HW [163.3 ± 20.2 (n = 3) vs. 140 ± 9.1 mg (n = 5)], BW (28.1 ± 3.02 vs. 25.5 ± 1.4 g), and HW-to-BW ratios (5.83 ± 0.51 vs. 5.49 ± 0.011) for α2δ-1 (−/−) and WT, respectively, were not significantly different.

Diminished cardiac contractility. We performed ex vivo working heart studies in WT (n = 6) mice and α2δ-1 (−/−) (n = 7) littermates (Table 2), hypothesizing that the deletion of the α2δ-1 subunit would result in a diminished contractility that was not dependent on altered vascular resistance or loading conditions. Indeed, when compared with WT, basal contractility and relaxation (+dP/dt and −dP/dt) were significantly lower in α2δ-1 (−/−) hearts.

Ca\(^{2+}\) channel properties. In whole cell patch-clamp measurements (I-V curves) on cardiomyocytes derived from WT and α2δ-1 (−/−) mice, the L-type VDCC \(I_{\text{Ca}}\) peak current density was significantly decreased by ~45%, compared with WT (\(I_{\text{Ca}}\) density; Fig. 5A, and Table 3). There was a significant increase in the half-activation potential \(V_{50}\) more than 4-fold) and the slope factor \(k\) (28%) between the WT and α2δ-1 (−/−) cardiomyocytes. Depolarizing pulses of different amplitudes were applied from a holding membrane potential of −50 mV. The inward currents reached their peak values at around +20 mV in the α2δ-1 (−/−) and +10 mV for WT (steady state activation) mice. There was a hypopolarizing shift in the voltage for half-maximal activation \(V_{50}\) right shift ~8.1 mV; Fig. 5B, and Table 3). The slope parameter \(k_{\text{activation}}\) value was not statistically different between the two groups. For the steady-state inactivation curves, the normalized amplitude of

Fig. 4. Hematoxylin and eosin slides of WT and −/− hearts.
the inward Ca\(^{2+}\) current evoked by the test pulse (+10 or +20 mV) was plotted as a function of prepulse voltage. The voltage of \(V_{0.5}\) was shifted to a hypopolarizing direction by \(\sim 13\) mV (Fig. 5C, and Table 3). The steepness of the inactivation curve \(k_{\text{inactivation}}\) did not change significantly between groups. The time course of the decay of \(I_{\text{Ca}}\) was analyzed by fitting a double exponential equation by Chebyshev. The fit interval started at 60 ms and ended at 440 ms. The time constant of the rapid component \(\tau_{\text{fast}}\) was significantly different depending on the pulse (+10 or +20 mV), suggesting the interference of a Ca\(^{2+}\)-dependent inactivation process, since Ca\(^{2+}\) was used as a charge carrier. The slow time constant \(\tau_{\text{slow}}\), representing voltage-dependent inactivation independent of current amplitude, was slowed in \(\alpha_\delta\)-1-deficient cardiomyocytes (Table 3). Cell capacitance was significantly decreased in \(\alpha_\delta\)-1 (-/-) cardiomyocytes (Table 3).

### DISCUSSION

The role of the accessory subunits on VDCC kinetics has been closely studied using heterologous coexpression systems. From these studies, it has been shown that both \(\alpha_\beta\)- and \(\beta\)-subunits increase membrane expression of the \(\alpha_1\)-subunit. The accessory subunits enhance current amplitude and density, as well as regulating the kinetics of activation and inactivation (5, 34, 38, 39). The coexpression of both accessory subunits appears synergistic, rather than additive, with respect to membrane expression of the \(\alpha_1\)-subunit and additive for the voltage-dependence of activation of the channel. In addition, whereas the \(\alpha_\delta\)-subunit increases Ca\(^{2+}\) channel density, the \(\beta\)-subunit both increases channel density and facilitates an
opening of the channel (38). However, since uninjected oo-
cytes express endogenous barium currents (28), as well as
endogenous α1C-, β-, and αδ/β-subunits (4, 29, 32), the studies
on regulatory functions of αδ/β- and β-subunits are inevitably
“contaminated” by these endogenous channels.

This is the first successful attempt at knocking out the
αδ/β-1-subunit in vivo. The genomic DNA PCR verified that
the integration of the targeting construct was at exon 2 of the
gene. This confirmed the results of the genotyping PCR.
Furthermore, the Western blot analysis and the binding data
clearly demonstrate the lack of αδ/β-1 protein in these animals.
The ablation of the αδ/β-1-subunit did not have any effect on
the expression levels of the other Ca2+

<table>
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<tr>
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<th>WT</th>
<th>n</th>
<th>αδ/β-1 (−/−)</th>
<th>n</th>
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<td>I_{Ca} density, pA/pF</td>
<td>5.76±0.30 (+10 mV)</td>
<td>35/5</td>
<td>3.17±0.19* (+20 mV)</td>
<td>47/7</td>
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<td>V_{0.5}, mV (I-V curve)</td>
<td>−2.32±1.18</td>
<td>27/5</td>
<td>4.66±1.14†</td>
<td>38/7</td>
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<td>k, mV (I-V curve)</td>
<td>5.29±0.17</td>
<td>8/3</td>
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<td>−3.50±1.15</td>
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<td>τ_{fast}, ms</td>
<td>19.90±1.25</td>
<td>23/5</td>
<td>44.74±3.67‡</td>
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<td>83.73±2.40</td>
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<td>138.92±13.39†</td>
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Cell capacitance, pF

222.25±11.84

Values are means ± SD; n, number of experiments performed (number of animals used to generate the myocytes/number of myocytes measured). I_{Ca} density, peak current density; I-V, current-voltage; V_{0.5} (I-V curve), half-activation potential; V_{0.5} (steady-state activation), half-maximal activation; V_{0.5} (steady-state inactivation), half-maximal inactivation; k, slope factor; τ_{fast}, time constant of the rapid component; τ_{slow}, slow time constant. *P < 0.05; †P < 0.001.

We have developed a knockout model that, while obvious-
ly having a profound affect on the regulation of the α_{1C}-subunit, is not lethal. It represents a novel model for
studying the function of the αδ/β-1-subunit and character-
izing its effects, particularly with respect to cardiovascular
function, epileptogenesis, and nociception, potentially lead-
ing to new pharmacological therapies.

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Genomics, University of Florida, College of Medicine, Gainesville, FL).

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