ONLINE DATA SUPPLEMENT

Expanded Materials and Methods

Generation of Knockout Mice

The targeting construct was prepared by ligating a 2.2-kb fragment from the 5’ end of the type 5 AC gene, which contains the exon with the first translation initiation site (5’-arm); a fragment containing a neomycin resistance gene fragment driven by a phosphoglycerate kinase promoter; and a 7.0-kb fragment of the type 5 AC gene (3’-arm) into pBluscript II KS (Stratagene) 1. Embryonic stem cells were transfected with 50 µg of linearized targeting vector by electroporation. Two clones were injected into C57BL/6 blastocysts, and chimeras were obtained. These chimeras successfully allowed germ-line transmission and were crossed with C57BL/6 females. F1-heterozygous offspring were then interbred to produce homozygous mutations. All mice were 129/SvJ-C57BL/6 mixed background littermates from F1 heterozygote crosses. All experiments were performed in 4-6 month old homozygous AC5−/− and wild type (WT) littermates. This study was approved by the Animal Care and Use Committee at New Jersey Medical School.

Radioligand Binding Assays and Western Blotting

Radioligand binding assays for β-AR were conducted using the above membrane preparations and 125I-cyanopindolol as previously described 2. Western blotting for type 5 AC, Gsα, Giα, Gqα, Gβγ, β1-adrenergic receptor (β1-AR), β-adrenergic receptor kinase (β-ARK), and muscarinic receptor type 2 were conducted using either the membrane preparation or whole tissue homogenates. Western blotting was conducted using commercially available antibodies, except for type 5 AC antiserum, which was
raised against the 27-mer amino acids (NH2-IGHNPPHWGAERPFYNHLGGNQVSKEC-COOH) that was obtained from the C1b domain of AC5 (amino acid residues 638-662). This sequence is conserved among different animal species but shows a very low sequence homology (14.8%) to the corresponding domain of the type 6 AC (kindly provided by Dr. James E Tomlinson, Millennium Pharmaceuticals Inc., Cambridge, MA). Because of its high specificity to type 5 AC, it does not cross react to type 6 AC. Accordingly, AC5^{-/-} showed a null, not reduced, expression of type 5 AC.

**Electrophysiological Studies**

Whole-cell currents were recorded using patch-clamp techniques as previously described \(^3\)\(^-\)\(^6\). Cell capacitance was measured using voltage ramps of 0.8 V/s from a holding potential of -50 mV. All experiments were performed at room temperature. Ca\(^{2+}\) channel currents (I\(_{Ca}\)) were measured with an external solution (mmol/L): CaCl\(_2\) or BaCl\(_2\) 2; MgCl\(_2\), 1; tetraethyl ammonium chloride, 135; 4-aminopyridine, 5; glucose, 10 and HEPES, 10 (pH 7.3). The pipette solution contained (mmol/L): Cs-aspartate, 100; CsCl, 20; MgCl\(_2\), 1; MgATP, 2; GTP, 0.5; EGTA, 5 or 1,2-bis (2-aminophenoxy)ethane-N, N', N', N''-tetraacetic acid (BAPTA), 10 and HEPES, 5 (pH 7.3). For potassium (K\(^+\)) channel current recordings, the external solution was normal Tyrode’s solution (mmol/L): NaCl, 135; CaCl\(_2\), 1.8; MgCl\(_2\), 1; KCl, 5.4; glucose, 10; HEPES, 10 (pH 7.3). Nifedipine (10 \(\mu\)mol/L) was added to block L-type Ca\(^{2+}\) channel currents. The patch pipette solution contained (mmol/L): potassium aspartate, 110; KCl, 20; MgCl\(_2\), 2; ATP, 2; GTP, 0.5; EGTA, 5; HEPES, 5 (pH 7.3).
References


Supplemental Figure 1 Legend

Comparison of histology in WT and AC5−/− heart. Note the normal architecture of the AC5−/− myocardium.
Supplementary Figure 1

WT

AC5

100 µm