SUPPLEMENTAL MATERIAL

Detailed Methods

Animals

Hearts were obtained from mice maintained in an inbred colony with C57BL/6J background (Jackson Laboratory, Bar Harbor, ME). Mice expressing GFP ($GFP^{+/-}$) [1] within the same C57BL/6J background were used as a reporter for $Cx43^{+/+}$ cells. $Cx43^{-/-}$ fetuses (Cx43KO) were obtained at embryonic day 20 (E20, 1 day before birth). Hearts from the $Cx43^{+/+}$-$GFP^{+/-}$ genotype (WT_{GFP}) were obtained within 24 h after birth (D1). As previously shown, there is no difference in electrical phenotype after 3-4 days of culture in cells obtained at E20 or D1. [2] The procedures involving the use genetically engineered animals and for cardiac excision complied with the rules of and were approved by the responsible agencies, the Bernese and the Swiss Veterinary Offices and the Swiss National Science Foundation.

Patterned Cell Cultures

The techniques to culture neonatal, murine cardiac myocytes on micropatterned strands have been described elsewhere.[2-3] Individual animals were genotpyed by PCR using standard protocols. After excision, the ventricles of individual hearts were minced, and the tissue fragments were dissociated in Hanks balanced salt solution devoid of Ca^{2+} and Mg^{2+} containing trypsin (0.2%, Roche Diagnostics) and pancreatin (120 μm/ml, Sigma). After enzymatic separation, cell types of different genotypes were preplated three times to eliminate fibroblasts. After genotyping, cells of the same genotype were pooled, cells were counted and cell suspensions were subsequently mixed to obtain defined ratios of Cx43KO and WT_{GFP} myocytes. Subsequently, the cell mixtures were seeded on fibronectin or collagen patterned coverslips. Patterns of cell pairs were obtained by standard soft microlithography techniques.[4-6] This technique involves PDMS (Polydimethylsiloxane, Dow Corning) coating of coverslips and subsequent transfer of extracellular matrix protein (fibronectin) patterns. Patterns of murine strands (4-5 mm in length and 50 or 200 μm in width) were obtained as described below. After seeding, the patterned cultures were kept in M199 medium (Hanks salts) supplemented with penicillin (20'000U/L, Biochrom), streptomycin (34μg/L, Biochrom), vitamin B12 (1.5 μm/L, Sigma), vitamin C (18 μm/L, Sigma), L-glutamine (136μm/L, Sigma), 5% neonatal calf serum (Amimed) and bromodeoxyuridine (100 μm/L, Sigma). For the first 24 hours epinephrine (10 μm/L, Sigma) was added. Experiments were carried out after 3-5 days in culture.

Whole cell dual voltage clamp

The classical whole cell dual voltage-clamp (DVC) method used to assess intercellular conductance, g_j, has been described previously in detail.[3] Patch electrodes (tip resistance 2-5 MΩ) were pulled from borosilicate glass () using a DMZ puller (Zeitz, Martinsried, Germany) and had an access resistance of 10.6±1.0 MΩ in accordance with previously reported values. [7] Transjunctional current, I_j, and junctional conductance, g_j, were measured using a HEKA10 amplifier (HEKA electronics, Germany). The dependence of steady-state junctional conductance on transjunctional voltage [$g_{j,ss}= f(V_j)$] was fit to $g_{j,ss}= f(V_j)$ used the Boltzmann equation applied separately to the data recorded at negative and positive V_j . [8] The true value for g_j was calculated from the measured g_j and the access resistance of the two electrodes according to van Rijen et al. [9]

Multisite High Resolution Optical Mapping of Transmembarne Potential

Optical recordings of action potentials and propagation velocities were obtained by high resolution optical mapping at a sampling frequency of 10 kHz using the optical-sensitive dyes

RH137 or di-8-ANEPPS. [2-3] The emitted light was collected by a custom-built hexagonal light guide, containing 380 fibers, placed in the focal plane of the microscope. The collected light from 128 fibers was measured by photodiodes (Hamamatsu, Switzerland), converted to voltage and pre-amplified by custom-built preamplifiers in a frequency range from 0 – 3.5 kHz. The spatial resolution was 25μm (40x objective) or 10μm (1000x objective). The signals were converted to digital information at a sampling rate of 12 kHz. Local activation time on the action potential upstrokes was defined as the time of change of membrane potential (V_m) at 50% of action potential amplitude (see {Kleber, 2004 #55}). Amplitudes of action potentials are expressed in relative units of % action potential amplitude. At an average action potential amplitude (%APA) of 100mV [10]), 1%APA corresponds to 1 mV.

Immunohistochemistry and confocal microscopy

The amounts of Cx43 and Cx45 at intercellular junctions were quantified by immunohistochemistry in paraformaldehyde-fixed cell preparations using antibodies and protocols described previously.[3, 6] The amount of immunoreactive signal at intercellular junctions was quantified using laser scanning confocal microscopy, image deconvolution and digital image processing algorithms (IMARIS software; Bitplane Inc, Zuerich, Switzerland) as validated in previous studies. [6, 11] Mouse myocyte cultures were fixed in 4% paraformaldehyde for 5 minutes and then incubated in blocking buffer (HBSS with 10% bovine serum albumin, 0.15% Triton X-100, 3% normal goat serum) for 30 minutes at room temperature. Cultures were incubated with mouse monoclonal anti-Cx43 antibodies (MAB 3068, Chemicon, Billerica, MA) and rabbit polyclonal anti-Cx45 antibodies (kindly provided by Dr. Kathryn Yamada, Washington University, St. Louis) overnight in a humid chamber at 4C. Cultures were then incubated with DAPI, tetramethylrhodamine goat anti-mouse antibodies, and Alexa Fluor 633 goat anti-rabbit antibodies (Invitrogen, Carlsbad, CA) for two hours at room temperature. Finally, cultures were incubated with Alexa Fluor 488 conjugate rabbit polyclonal anti-GFP antibodies (A-21311, Invitrogen, Carlsbad, CA) for two hours at room temperature. Coverslips were then mounted on a glass slide with ProLong Gold antifade reagent (Invitrogen, Carlsbad, CA).

Statistics

Results are expressed as mean values ± S.E. ANOVA and non-paired Student's t-tests were used to calculate statistical significances ($p < 0.05\%$).

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