Ketamine inhibits synaptic transmission and nicotinic acetylcholine receptor-mediated responses in rat intracardiac ganglia in situ

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Supplementary Material

Ketamine inhibits synaptic transmission and nicotinic acetylcholine receptor-evoked responses in rat intracardiac ganglia *in situ*

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Supplementary Methods

Electrophysiology

HEK293T cells (ATCC, USA) were cultured in DMEM (Invitrogen Life Technologies, Australia) supplemented with 10% FBS (Bovigen, Australia), 1% penicillin and streptomycin and 1x GlutaMAX (Invitrogen Life Technologies) at 37°C in 5% CO_2. HEK293T cells were plated on 12 mm glass coverslips and transiently transfected using the calcium phosphate method. Plasmid cDNAs encoding human Cav2.1 (provided by Dr J. Striessnig, University of Innsbruck, Innsbruck, Austria), Cav2.2 (provided by Dr D.T. Yue, The John Hopkins University School of Medicine, Baltimore, MS, USA) or Cav2.3 (provided by Dr T. Schneider, University of Cologne, Cologne, Germany) were co-transfected with human α2δ1 and β3 together with a green fluorescent protein (GFP) expressing plasmid (for identification of transfected cells).

Whole-cell patch clamp recordings were performed within 24-48 hours post-transfection at room temperature (21-23°C). Cells were constantly superfused with extracellular solution containing (in mM): 100 NaCl, 10 $CaCl_2$, 1 $MgCl_2$, 5 CsCl, 30 TEA-Cl, 10 Glucose, 10 HEPES (pH 7.35 with TEA-OH; ~310 mOsmol·kg^{-1}). Fire-polished borosilicate pipettes (1-3 MΩ) were filled with intracellular solution containing (in mM): 140 K-Gluconate, 5 NaCl, 2 $MgCl_2$, 5 EGTA, 10 HEPES (pH 7.2 with KOH; ~290 mOsmol·kg^{-1}). Calcium currents were elicited by a test depolarization to +30 mV (50 ms duration) from a holding potential of −90 mV at 0.1 Hz. Use of ketamine HCl (Ketamil) was approved by NSW Ministry of Health (Approval #A-201911-74).

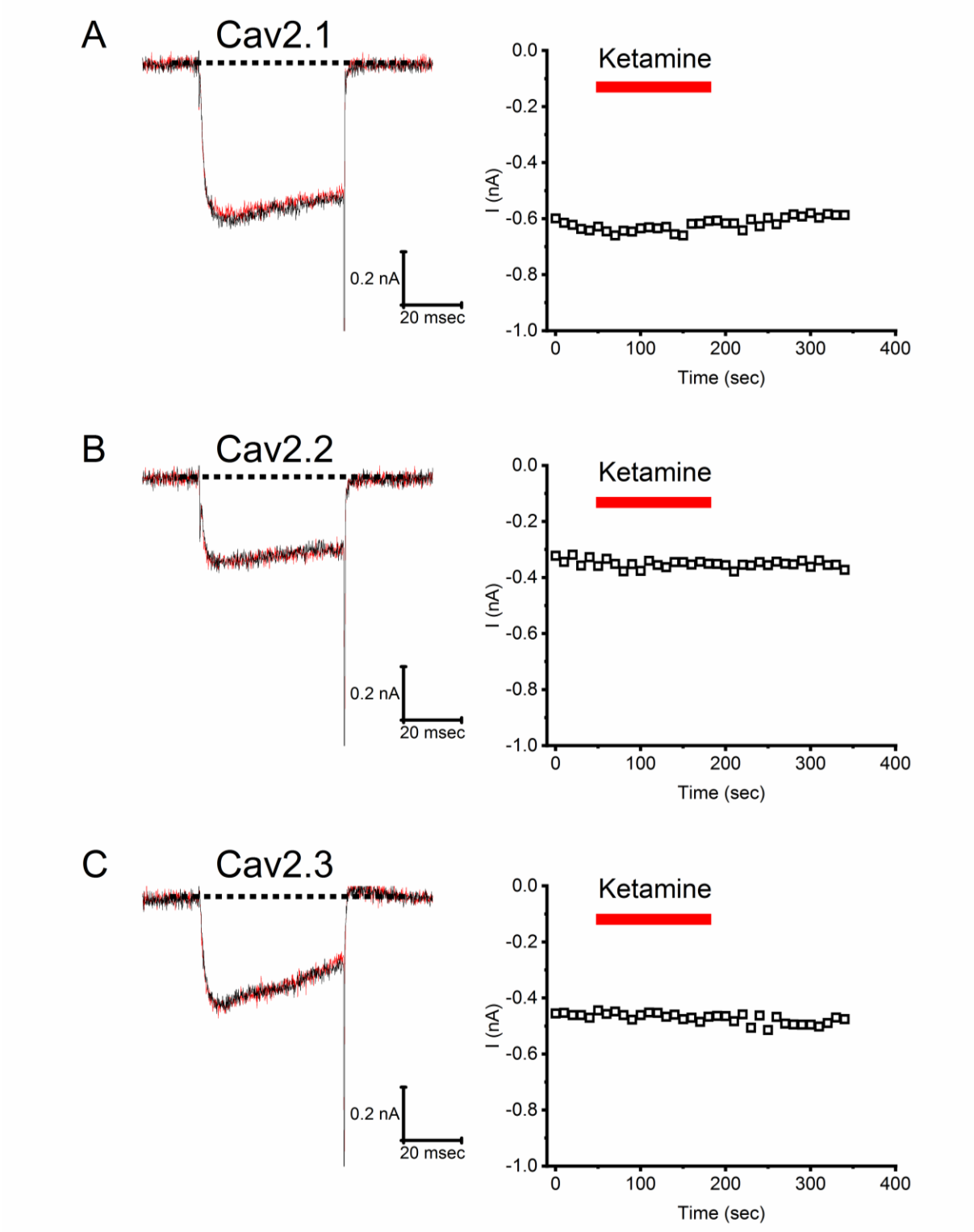

Figure S1. Ketamine (100 µM) does not inhibit human Cav2.1, Cav2.2 or Cav2.3 channels heterologously expressed in HEK293T cells. Representative depolarization-activated Ca^{2+} currents (right) elicited from a holding potential of −90 mV to a test potential of +30 mV (50 msec duration; 0.1 Hz) from Cav2.1 (A), Cav2.2 (B) and Cav2.3 (C) in the absence (control, black) and presence of 100 µM ketamine (red). Corresponding timeplots of Ca^{2+} current amplitude before, during and upon washout of 100 µM ketamine application for each Cav are shown in the LHS panels.