Role of T-type calcium channel subunits in post-myocardial infarction remodelling probed with genetically engineered mice

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Aims

Previous studies suggested that T-type Ca\(^{2+}\)-current (I\(_{CaT}\))-blockers improve cardiac remodelling, but all available I\(_{CaT}\)-blockers have non-specific actions on other currents and/or functions. To clarify the role of I\(_{CaT}\) in cardiac remodelling, we studied mice with either of the principal cardiac I\(_{CaT}\)-subunits (Cav3.1 or Cav3.2) knocked out.

Methods and results

Adult male Cav3.1- or Cav3.2-knockout (Cav3.1\(^{-/-}\), Cav3.2\(^{-/-}\)) mice and respective wild-type (WT) littermate controls were subjected to left anterior descending coronary artery ligation to create myocardial infarction (MI). Echocardiography and programmed electrical stimulation were performed at baseline and 4 weeks post-MI. At baseline, Cav3.1\(^{-/-}\) mice had slowed heart rates and longer PR intervals vs. WT, but no other electrophysiological and no haemodynamic differences. Cav3.2\(^{-/-}\) showed no differences vs. WT. Contractile indices (left ventricular fractional shortening and ejection fraction) decreased more post-MI in Cav3.1\(^{-/-}\) mice than in Cav3.1\(^{+/+}\) (e.g. by 34 and 29% for WT; 50 and 45% for Cav3.1\(^{-/-}\), respectively; \(P < 0.05\) for each). Cav3.1\(^{-/-}\) mice had increased ventricular tachycardia (VT) inducibility post-MI (9 of 11, 82%) vs. WT (3 of 10, 30%; \(P < 0.05\)). Cav3.2\(^{-/-}\) mice were not different in cardiac function or VT inducibility vs. WT. Quantitative polymerase chain reaction showed that Cav3.1 is the major I\(_{CaT}\)-subunit and that no compensatory Cav3.2 up-regulation occurs in Cav3.1\(^{-/-}\) mice. Cav3.1\(^{-/-}\) and Cav3.2\(^{-/-}\) mice had no mRNA expression for the knocked-out gene, at baseline or post-MI.

Conclusion

Our findings suggest that, contrary to suggestions from previous studies with (imperfectly selective) pharmacological agents having T-type Ca\(^{2+}\)-channel-blocking actions, elimination of Cav3.1 expression leads to impaired cardiac function and enhanced arrhythmia vulnerability post-MI, whereas Cav3.2 elimination has no effect.

Keywords

Ca-channel • Gene expression • Infarction • Remodelling

1. Introduction

There are two principle types of Ca\(^{2+}\)-channels in the heart, ‘long-lasting’ L-type channels, and ‘transient’ T-type channels.1,2 L-type Ca\(^{2+}\)-current (I\(_{CaL}\)) has well-understood roles in cardiac impulse initiation and propagation, arrhythmogenesis, and contraction. The function of T-type Ca\(^{2+}\)-current (I\(_{CaT}\)) is less well understood. T-type channels clearly participate in sinus-node pacemaker function and in atrioventricular-node conduction.3 In addition, there is evidence for a deleterious role of I\(_{CaT}\) in cardiac remodelling and arrhythmogenesis.1 I\(_{CaT}\) is strongly expressed in foetal hearts and its expression is greatly reduced in the mature heart; however, cardiac hypertrophy restores I\(_{CaT}\) expression to substantial levels.4 Neonatal cardiomyocyte hypertrophy is induced by hyperglycaemia, concomitant with enhanced I\(_{CaT}\) expression.5 Suppression of I\(_{CaT}\) with mibebradil or nickel prevents cell proliferation changes.5

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The I_{CaT}-selective blocker mibebradil improves myocardial remodelling in a variety of paradigms. Mibebradil has been reported to suppress remodelling and improve haemodynamic function in a rat myocardial infarction (MI) model, prevent exercise-induced myocardial stunning in dogs, and abolish atrial tachycardia-induced arrhythmogenic remodelling in dogs. Comparative studies have shown greater efficacy of mibebradil vs. I_{CaL}-selective inhibitors in post-MI functional and Ca^2+-handling preservation, in preventing myocardial damage following acute ischaemia and reperfusion in isolated hearts from normal rats and from guinea pigs with acute renal failure, and in preventing arrhythmogenic remodelling by chronic atrial tachycardia in dogs. On the other hand, possible deleterious effects of mibebradil have been reported in post-MI remodelling and on contractile function in hypertrophied hearts. A recent study suggested that the I_{CaT}-blockers efonidipine and mibebradil prevent sudden death in mice with heart failure, whereas the I_{CaL}-blocker nitrendipine does not.

A major difficulty in establishing the functional role of T-type Ca^2+ channels has been the limited selectivity of pharmacological probes. The establishment of the molecular identity of I_{CaT} α-subunits has permitted the development of genetically engineered mice, allowing for clarification of the functional role of I_{CaT}. Mice with increased I_{CaT} due to Cav3.1-subunit overexpression have smaller increases in contractility and no enhanced sarcoplasmic reticulum (SR) Ca^2+ load compared with mice with increased I_{CaL} due to overexpression of the L-type channel β-subunit. Nakayama et al. have shown that Cav3.1 overexpression abrogates, whereas Cav3.1 knockout enhances, overload-, isoproterenol-, and exercise-induced cardiac hypertrophy by a nitric-oxide synthase-dependent mechanism. However, other workers demonstrated that pressure-overload hypertrophy is suppressed in Cav3.2-knockout mice (KO) and unaffected by Cav3.1 deletion. Therefore, the role of I_{CaT} and of its respective subunits in adverse cardiac remodelling remains unclear. In the present study, we addressed this issue by assessing the effects of Cav3.1 and Cav3.2 knockout in mice subjected to acute MI, a highly clinically relevant form of cardiac remodelling.

2. Methods

2.1 Cav3.1^{+/−} and Cav3.2^{+/−} mouse lines

Cav3.1 and Cav3.2 mouse lines were obtained in C57BL6/SV129RvEvBrd strains from Lexicon Genetics. Heterozygous mice were used for breeding and homozygous KO and wild-type (WT) littermates were used for comparison. Briefly, exon-1 for Cav3.1 or Cav3.2 was deleted and replaced by IRES-lacZ/MC1-Neo for selection in pKOS vectors. Embryonic stem (ES) cells were transfected with the KO insert and recombinant ES cells were selected with the appropriate antibiotic. Recombinant ES cells were then injected back into mouse embryos and mice missing the gene of interest were selected by polymerase chain reaction (PCR). Ear-clip samples were digested with proteinase-K at 50°C for 20 h, and genomic DNA was then purified with isopropanol precipitation followed by washing with 70% ethanol. Genotyping after each breeding was performed with the following primers: Cav3.1: forward (5'-GGTTGTGTGGAGGACACTC), reverse (5'-GGTTGACTCTGTAAGGCT), yielding a 145 bp WT product; forward (5'-GCAGCGCATCGCCTTCTATC), reverse (5'-CCGATGCGAAGCTCTGA), generating a 277 bp KO product. PCR was performed with Taq Polymerase (Invitrogen); annealing temperature 54°C, PCR run for 35 cycles.

2.2 Animal handling

All experimental protocols were approved by the local Animal Research Ethics Committee and conducted according to the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996). Adult male KO and WT mice (30–35 weeks old at entry) were used in this study. The initial surgical procedure for MI creation was performed under aseptic conditions. Mice were anaesthetized with 2.5% isoflurane (absence of response to painful stimuli used as the index of adequate anaesthesia) via the endotracheal intubation used for continuous mechanical ventilation with parameters set according to a nomogram. A surface ECG (lead I) was recorded for monitoring and later off-line analysis. Body temperature was kept at 37°C with a homoeothermic warming blanket. A 1.5 cm vertical left parasternal skin incision was then made 2 mm away from the left sternal border and the chest was opened at the fourth inter-costal space. After removing the pericardial sac and slightly retracting the left auricle, the left anterior descending coronary artery (LAD) was indented and ligated 2 mm below the tip of the left auricle. Mice were treated with the long-acting narcotic analgesic drug buprenorphine (Temgesic, 500 IU/kg, ip) 20 min before LAD ligation.

2.3 Electrocardiogram and telemetry recording

At baseline and 4 weeks post-MI, mice were anaesthetized for ECG recording with 2.5% isoflurane. Body temperature was maintained at 37°C with a heating pad (Harvard Apparatus, USA). A surface ECG (lead I) was obtained with three 25-gauge subcutaneous electrodes and transmitted to a computer via an analogue-digital converter (IOX v1.585, EMKA Technologies) for monitoring and later analysis with ECG-Auto 1.5.12.10 software (EMKA Technologies). Recordings were filtered between 0.3 and 500 Hz. Measurements were based on averages of 10 consecutive complexes. Standard criteria were used to measure RR, PR, QR, QT intervals. The QT interval was corrected for heart rate (HR) as follows: corrected QT interval (QTc) = QT/√RR100. With QT and RR, expressed in milliseconds (ms).

To obtain 24 h ECG recordings after LAD ligation in free-moving mice, mice were anaesthetized 1 week before LAD ligation with 2.5% isoflurane delivered via a ventilator. An abdominal incision was made 1.5 cm lateral to the midline to insert a telemetry transmitter (TA10EA-F20, Data Sciences International) into a subcutaneous pocket with paired wire electrodes placed over the thorax (to obtain a bipolar chest ECG lead). Mice were housed in individual cages with free access to food and water and were exposed to 12 h light/dark cycles (light, 6:00 AM to 6:00 PM) in a thermostatically controlled room. ECG signals were computer recorded with the use of a telemetry receiver (RPC1, DSI International), and IOX 2.3 and ECG-Auto 2.4.0.30 (EMKA) systems were used for display and analysis. HR values were determined from average RR intervals over 10 s.

2.4 Intra-cardiac recording and pacing

At baseline and 4 weeks after LAD ligation, mice were anaesthetized with 2.5% isoflurane. An octopolar electrophysiology catheter (1.2F) designed for mouse electrophysiology (Biosense Inc.) was positioned in the right ventricle via the right internal jugular vein. Intra-cardiac electrogrograms were used as a guide for catheter positioning. Surface ECG (lead I) and intra-cardiac electrogrograms were recorded on a computer through an analogue-digital converter (IOX 1.11, EMKA Technologies) for monitoring and later analysis (ECG-Auto 2.14.4.15). Intra-cardiac electrogrograms were filtered between 0.5 and 500 Hz. Pacing was performed with a custom-built computer-based stimulator triggering a stimulus isolator (509 Stimulator, Grass Telefactor). Standard pacing protocols were used to determine electrophysiological parameters as described elsewhere. Refractory
periods were determined with a nine-stimulus drive train (S1) at a cycle length of 100 ms followed by a 1.5 × threshold-current premature stimulus (S2) progressively decremented in 2 ms intervals. Atrial, atrioventricular nodal, and ventricular effective refractory periods (AERP, AVERP, and VERP, respectively) were defined as the longest S1-S2 coupling interval that failed to generate a propagated beat. Programmed single, double, and triple extrastimuli were applied at a 100 ms drive cycle length. Burst pacing consisted of trains of 50 paced beats at a cycle length from 50 to 15 ms, with at least a 4 s interval between two successive trains (unless arrhythmias were induced). Ventricular tachycardia (VT) was defined as 10 or more successive spontaneous ventricular beats. Atrial fibrillation (AF) was defined as a rapid and irregular atrial rhythm with variable ventricular response, and was considered sustained if it lasted >1 s before termination.

2.5 Echocardiography

Transthoracic echocardiography was performed under 2.5% isoflurane anaesthesia before LAD ligation at baseline, and 4 weeks following MI, using an 13L (10–14 MHz) probe and a Vivid-7 Dimension system (GE Healthcare Ultrasound, Horten, Norway). The left ventricular (LV) para-sternal long-axis view was used to measure the dimension (D) of the LV outflow tract (LVOT). LVOT cross-sectional area (CSALVOT) was calculated as CSALVOT = π(D/2)^2. The LV short-axis view at the papillary muscle level was recorded: LV areas at both cardiac end-diastole (EDA) and end-systole (ESA) were measured with the papillary muscle excluded, along with the LV anterior wall and posterior wall thickness at end-diastole. LV fractional area change was calculated as [(EDA − ESA)/EDA] × 100%. LV M-mode images at this level were used to determine LV dimensions at end-diastole (LVDd) and end-systole (LVDs). LV fractional shortening (FS) was calculated as [(LVDd − LVDs)/LVDd] × 100%. The Teichholz method was employed to calculate LV volumes and LV ejection fraction (EF). An apical five-chamber view was used to obtain the velocity-time integral of trans-LVOT flow (VTILVOT) with a pulsed-wave Doppler proximal to the aortic valve. HR was determined on a simultaneously recorded ECG. Stroke volume (SV) was calculated as SV = VTILVOT × CSALVOT, and cardiac output was calculated as CO = HR × SV. The average of three consecutive cardiac cycles was used for each measurement. Special care was taken to obtain similar imaging planes at baseline and follow-up studies. The operator was blinded to genotype.

2.6 Haemodynamic indices

Haemodynamic indices were obtained following catheterization under 2.5% isoflurane anaesthesia. Body temperature was maintained at 37°C. A micro-tip Millar-type pressure-volume 1.2F catheter (Scisense Inc., Canada) was inserted into the right carotid artery and advanced into the LV. Correction of the conductance was performed by injecting 5 μL of 20% NaCl into the left jugular vein. At baseline and 4 weeks post-MI, the mean blood pressure, the maximal (+dP/dt) and minimal (−dP/dt) first derivatives of LV pressure, the contractility index, and the relaxation time were calculated with IOX v1.8.9.4 software (EMKA). After the haemodynamic measurements, mice were euthanized by cervical dislocation under 2.5% isoflurane anaesthesia, and the heart was removed and washed in Ca2+-free Tyrode solution. The transmural-MI scar was removed and weighed.

2.7 Taqman real-time quantitative PCR

The left atrium (LA) and LV were flash-frozen in liquid-N2 for subsequent RNA isolation. Procedures for the Taqman real-time quantitative polymerase chain reaction (qPCR) followed previously described methods.21 Total RNA was isolated using the Trizol method (Invitrogen). Total RNA was then treated with DNase I (Sigma Chemicals) to prevent genomic DNA contamination. Lack of genomic DNA contamination was confirmed by PCR. The quality of RNA was assessed with a bioanalyzer (Agilent 2100). Total RNA was reverse-transcribed with the high-capacity reverse transcription kit (Applied Biosystems). qPCR was performed with TaqMan probes and primers from Applied Biosystems on the T-type calcium channel genes: Cav3.1 (Assay ID: Mm00486549_m1), Cav3.2 (Assay ID: Mm00445369_m1). The geometric mean of HPRT1 (Assay ID: Mm00469686_m1) and GAPDH (Assay ID: Mm99999915_g1) was used for normalization. qPCR reactions were performed with the Taqman Gene Expression Master Mix kit (Applied Biosystems). Reactions were run on a Stratagene MX3000. Standard curves were generated for each set of primers over a 2-log range and the efficiencies were 100 ± 5%. Relative gene-expression values were calculated by the 2−ΔΔCt method.21

2.8 Data analysis

Data are expressed as mean ± SEM. Unpaired t-tests were used to compare single non-repeated means from different groups and for multiple measures, ANOVA followed by Bonferroni-corrected t-tests or Tukey tests. Fisher’s exact test was used to analyse categorical variables. A two-tailed P-value of <0.05 denoted statistically significant differences.

3. Results

Detailed results in both groups for ECG, electrophysiological, echocardiographic, haemodynamic, and heart-weight analyses in all mice at baseline and 4 weeks post-MI are tabulated in Supplementary material online, Tables S1 and S2. Results of particular relevance are presented in figures and described below.

3.1 Electrocardiographic recordings, hypertrophic, and infarct-size indices

Examples of typical ECG recordings are shown in Figures 1A–D, and mean data in Figure 1E–H. RR (Figure 1E) and PR (Figure 1F) intervals were significantly increased in Cav3.1−/− mice compared with Cav3.1+/+, both at baseline and 4 weeks post-MI, consistent with previous observations.3 Cav3.1+/+ and Cav3.1−/− mice showed no significant differences in the QRS interval (Figure 1G) or QTc (Figure 1H), P-wave duration (see Supplementary material online, Table S1), or QTc interval (Figure 1D). Cav3.2−/− mice had no changes in any ECG index (see Supplementary material online, Table S2). MI significantly prolonged QRS and QTc intervals, with similar changes in all KO and WT groups.

The mortality 7 days post-MI was similar for Cav3.1−/− and Cav3.1+/+ mice (16 of 39, 41%, WT vs. 22 of 46, 48%, KO, P = NS). The ratio of total ventricular weight (TVW) over body weight (BW), an index of cardiac hypertrophy, was increased in both groups 4 weeks post-MI (Figure 2A), without intergroup differences. Similarly, cell capacitance increased post-MI (see Supplementary material online, Figure S1), reflecting cellular hypertrophy, but Cav3.1−/− and Cav3.1+/+ mouse changes were similar. Furthermore, the infarct size/TVW ratio was not different between Cav3.1+/+ and Cav3.1−/− mice (Figure 2B), excluding infarct-size extension or reduction as a mediator of any changes. Similar results were observed in Cav3.2 mice, with no significant difference in post-MI mortality between groups (14 of 32, 44%, Cav3.2+/+ vs. 19 of 36, 53%, Cav3.2−/−, P = NS). The TVW/BW ratio increased in both Cav3.2+/+ and Cav3.2−/− mice 4 weeks post-MI vs. baseline (Figure 2C); however, no significant difference was observed between the two groups. The infarct size was not different between Cav3.2+/+ and Cav3.2−/− mice (Figure 2D).
3.2 Cardiac contractility and haemodynamics

LV FS (Figure 3A) and EF (Figure 3B) were not different in Cav3.1\(^{-/-}\) mice compared with Cav3.1\(^{+/+}\) mice at baseline. MI decreased LV FS and EF significantly in both groups. However, both FS and EF were more strongly decreased by MI in Cav3.1\(^{-/-}\) mice (by 50 and 45% respectively) than Cav3.1\(^{+/+}\) mice (by 34 and 29%, respectively; \(P < 0.05\) for each). The LV contractility index was also significantly smaller in Cav3.1\(^{-/-}\) vs. Cav3.1\(^{+/+}\) mice at 4 weeks post-MI (Figure 3C), but there were no significant differences in the mean blood pressure (Figure 3D). The LV contractility index was also significantly smaller in Cav3.1\(^{-/-}\) vs. Cav3.1\(^{+/+}\) mice at 4 weeks post-MI, but there were no significant differences in the mean blood pressure (Figure 3D). There were no significant differences in LV FS (Figure 3E), EF (Figure 3F), contractility index (Figure 3G), or blood pressure (Figure 3H) values of Cav3.2\(^{-/-}\) vs. Cav3.2\(^{+/+}\) mice, either at baseline or post-MI. Post-MI haemodynamic impairment in Cav3.1\(^{-/-}\) mice was also evidenced by significantly greater abnormalities in cardiac index and relaxation time (see Supplementary material online, Table S1). Other indices of cardiac performance, such as maximum positive and negative LV pressure generation (\(\text{maxd}P/\text{d}t\) and \(\text{mind}P/\text{d}t\)), end-diastolic pressure (\(P_{ed}\)), end-systolic pressure (\(P_{es}\)), time constant of relaxation (\(\text{Tau}\)), or stroke volume (\(SV\)) were not significantly different between Cav3.1\(^{-/-}\) and Cav3.1\(^{+/+}\) mice (see Supplementary material online, Table S2).

3.3 Cardiac electrophysiology and arrhythmias

Spontaneous ventricular tachyarrhythmias (VTs) were sometimes seen in the first 24 h post-MI (Figure 4A). During the 24 h post-MI telemetry period, spontaneous VT occurred in 2 of 7 Cav3.1\(^{+/+}\) mice and 2 of 6 Cav3.1\(^{-/-}\) mice (Figure 5A, \(P = \text{NS}\)). VTs were also inducible in some mice during electrophysiological study (Figure 4B), both at baseline assessment and 4 weeks post-MI. The prevalence of reproducible VT induction (Figure 4B) was not significantly different at baseline, occurring...
in 1 of 8 Cav3.1−/− mice (vs. 0 of 9 Cav3.1+/+ mice). However, at 4 weeks post-MI, VT was inducible in 9 of 11 (82%) Cav3.1−/− mice, but only in 3 of 10 (30%) Cav3.1+/+ mice (P < 0.05). VT duration (692 ± 113 ms Cav3.1+/+; 607 ± 160 ms Cav3.1−/−) and cycle length (47.3 ± 4.1 ms Cav3.1+/+; 52.0 ± 3.6 ms Cav3.1−/−) did not differ between groups. The inducibility of sustained AF was not significantly different between Cav3.1+/+ and Cav3.1−/− mice at either baseline (0 of 9 vs. 0 of 8 mice, respectively) or 4 weeks post-MI (2 of 10 vs. 3 of 11 mice). Mean AF durations were also not significantly different: 1.3 ± 0.6 vs. 1.9 ± 0.9 s for Cav3.1+/+ vs. Cav3.1−/− mice, respectively, at 4 weeks post-MI. No significant differences in AERP (Figure 5C) or VERP (Figure 5D) were observed between Cav3.1−/− and Cav3.1+/+ mice. In Cav3.1−/− mice, the AH interval was significantly longer compared with Cav3.1+/+ mice at baseline as well as at 4 weeks post-MI (see Supplementary material online, Table S1), indicating that their longer PR interval was due to abnormal atrioventricular (AV)-node function. Significant differences were also observed in the corrected sinoatrial (SA)-node recovery time at baseline and Wenckebach cycle length at baseline and 4 weeks post-MI (see Supplementary material online, Table S1), in agreement with ECG indicators of abnormal SA- and AV-node function. Consistent with the absence of QRS differences between Cav3.1+/+ and Cav3.1−/− mice, no significant differences were observed in the HV interval. Cav3.2−/− and Cav3.2+/+ mice showed no significant electrophysiological differences (see Supplementary material online, Table S2).

### 3.4 Cav3.1 and Cav3.2 gene expression

Cav3.1 and Cav3.2 gene expression quantification data are shown in Figure 6. Cav3.1 was undetectable in both LA and LV at baseline and post-MI in Cav3.1−/− mice (Figure 6A). Cav3.2 expression (Figure 6B) was lower than that of Cav3.1 (Figure 6A) in Cav3.1+/+ mice, confirming previous observations indicating that Cav3.1 is the dominant subunit in the mouse.21 Cav3.1 expression did not change significantly post-MI (Figure 6A and C) in Cav3.1+/+ and Cav3.2+/+ mice, and Cav3.2 expression actually decreased post-MI in Cav3.1+/+ mice (Figure 6B). Cav3.1 expression was not altered in Cav3.2−/− KO (Figure 6C). Cav3.2 was undetectable at baseline and post-MI in Cav3.2−/− mice (Figure 6D).

### 4. Discussion

In this study, we examined the response of Cav3.1−/− and Cav3.2−/− mice to post-MI remodelling. We found that, contrary to expectations based on previous pharmacological studies with I_{CaT}-blockers, post-MI remodelling was not improved by I_{CaT}-subunit deletion. In the case of Cav3.1, post-MI remodelling was worsened, and in the case of Cav3.2 it was unaltered.

#### 4.1 Comparison with previous studies of effects of I_{CaT} inhibition on cardiac remodelling

A variety of studies used the I_{CaT}-selective blocker mibefradil to assess effects of I_{CaT} inhibition on remodelling. Min et al.3 observed improved haemodynamics in post-MI rats treated with mibefradil, compared with minor effects of amlodipine and verapamil. Sandmann et al.4 noted reduced post-MI fibrosis as well as improved haemodynamics with mibefradil, with the greatest benefit observed when treatment began pre-infarction. Mozaffari et al.5 found that mibefradil was particularly effective in attenuating the effect of high after load on infarct size. Two studies indicated greater protection by mibefradil vs. I_{CaL}-selective
Figure 3  Haemodynamic indices for WT and KO mice at baseline and 4 weeks (4-w) post-MI. Mean ± SEM LVFS (A and E), EF (B and F), contractility index (C and G), and mean systemic arterial pressure (D and H) of Cav3.1+/+ vs. Cav3.1−/− mice (Top panels) and Cav3.2+/+ vs. Cav3.2−/− mice (Bottom panels). Results for all haemodynamic and echocardiographic indices are provided in Supplementary material online, Table S1 (Cav3.1 mice) and Supplementary material online, Table S2 (Cav3.2 mice). *P < 0.05, **P < 0.01, ***P < 0.001, ANOVA with Bonferroni post-tests. (n = 9, 7 for Cav3.1+/+, Cav3.1−/− and n = 6, 5 for Cav3.2+/+, Cav3.2−/−, respectively).

Figure 4  Examples of ventricular tachyarrhythmias post-MI. (A) Spontaneous monomorphic VTs recorded by ambulatory telemetry in an awake, unrestrained Cav3.1−/− mouse post-MI. (B) VT induced by programmed electrical stimulation in a Cav3.1−/− mouse under general anaesthesia 4 weeks post-MI. Top: ECG lead-II; bottom: intra-cardiac electrogram (Endo.). A train of nine basic (S1) stimuli was applied at a 100 ms cycle length, followed by two extrastimuli (S2 and S3).
blockers against ischaemic damage in Langendorff-perfused hearts.\textsuperscript{10,11} Mibefradil was also found to prevent exercise-induced myocardial stunning in dogs,\textsuperscript{7} glucose-induced neonatal-rat cardiomyocyte proliferation,\textsuperscript{5} and atrial tachycardia-induced atrial electrical remodelling.\textsuperscript{12}

Not all studies have pointed to benefits from I\textsubscript{CaT}-blockade. Xia et al.\textsuperscript{13} noted that ramipril improved post-MI remodelling in stroke-prone spontaneously hypertensive rats, but that mibefradil was not protective and even increased LV end-diastolic pressures. Takebayashi et al.\textsuperscript{14} found deleterious effects on right-ventricular twitch tension in rats exposed to monocrotaline to induce pulmonary hypertension and right-ventricular hypertrophy.

In the present study, we used a genetic-engineering approach to suppress ICaT by specifically knocking out the T-type Ca\textsuperscript{2+} channel subunits Cav3.1 and Cav3.2. We saw no evidence of protection against post-MI remodelling with ICaT-a-subunit deletion. On the contrary, Cav3.1-KO mice developed greater post-MI impairments in myocardial function and more arrhythmia susceptibility than WT mice. The discrepancy from previous results obtained with mibefradil as a pharmacological probe may relate to the limited specificity of mibefradil. In addition to inhibiting ICaT, mibefradil also suppresses ICaL,\textsuperscript{23} INa,\textsuperscript{24} IKr,\textsuperscript{25} and cytochrome function,\textsuperscript{26} any of which could have contributed, along with presently unrecognized actions of the compound, to its effects.

### 4.2 Recent insights into T-type Ca\textsuperscript{2+}-channel function from genetically engineered mouse models

The ability to produce mice that are genetically engineered to lack Cav3-channel subunits is providing new insights into the functional role of ICaT. Mangoni et al.\textsuperscript{5} showed that ICaT knockout impairs sinus-node automaticity and atrioventricular-node conduction, indicating an important role in nodal function. Jaleel et al.\textsuperscript{16} studied the effects of ICaT augmentation by Cav3.1 overexpression and compared them with the effects of ICaL enhancement by overexpression of the L-type Ca\textsuperscript{2+}-channel b2a-subunit. Although Ca\textsuperscript{2+} influx was increased similarly in both models, enhanced SR Ca\textsuperscript{2+} loading only occurred with ICaL enhancement and Cav3.1 overexpression produced smaller contractile enhancement. Nakayama et al.\textsuperscript{17} recently examined the effect of Cav3.1 overexpression and knockout on various forms of cardiac hypertrophy. They noted exaggerated hypertrophic remodelling with Cav3.1 knockout and reduced hypertrophy with Cav3.1 enhancement. In contrast, Chiang et al.\textsuperscript{18} did not observe an effect of Cav3.1 knockout on pressure-overload hypertrophy, but identified an important role for Cav3.2-subunits in the hypertrophic response to pressure overload, with the presence of Cav3.2-subunits being essential for calcineurin/nuclear factor of activated T-cells hypertrophic signalling to occur. Consistent with the findings of Nakayama et al., and different from those of Chiang et al., we found that Cav3.1 knockout enhanced adverse cardiac remodelling, whereas Cav3.2 knockout had no effect.

### 4.3 Novelty and potential significance

Our study is the first to test the effects of specific T-type ICaT suppression by genetic engineering on the remodelling response to acute MI. We found that, contrary to expectations from most previous pharmacological studies, ICaT suppression is not beneficial against post-MI remodelling, and may in fact be harmful. Adverse myocardial remodelling is an important determinant of outcome after acute MI.\textsuperscript{27} There is...
presently great interest in developing novel therapeutic targets for post-MI remodelling intervention. Based on the available pharmacological data, ICaT appears to be an interesting target. The prototype agent mibepradil was withdrawn because of off-target inhibitory effects on cytochromes that caused potentially disastrous pharmacokinetically based drug interactions. The pharmaceutical industry has been working actively to develop novel T-type Ca\textsuperscript{2+}-channel blockers with improved selectivity and safety. One of these, efonidipine, has been shown to have beneficial effects on myocardial remodelling. It has slightly more T/L-channel-blocking selectivity than mibepradil and has also been shown to have beneficial effects on myocardial remodelling. Our results suggest that ICaT blockade that targets Cav3.1-subunits may not be a beneficial property and should perhaps be avoided in the search for increasingly selective compounds. Whether targeting other ICaT-subunits like Cav3.2 is beneficial for other forms of remodelling, such as that caused by AF, remains to be determined.

Our work complements recent studies of ICaT participation in hypertrophic mouse models. These studies obtained contradictory information about the role of Cav3.1-subunits. Our observations support the notion that the absence of Cav3.1-subunits exaggerates adverse cardiac remodelling, and argue against negative consequences of Cav3.2 knockout. Our work extends the available knowledge base to the clinically important context of post-MI remodelling, about which there had been no information from mouse models engineered to lack ICaT-subunits. Interestingly, we did not observe any differences in ventricular weight or ventricular weight/BW ratios among groups, suggesting that Cav3.1\textsuperscript{-/-}-associated adverse remodelling was not associated with changes in cardiac hypertrophic responses.

### 4.4 Potential limitations

Although this study revealed deleterious effects of Cav3.1 but not Cav3.2 knockout on post-MI ventricular remodelling, we did not establish the mechanistic basis of these interesting findings. The most likely explanation is that increased Cav3.1-channel function is part of the adaptive response to the contractile impairment caused by loss of myocardial tissue due to infarction. Jaleel et al. have demonstrated to be part of the hypertrophic response of the heart. We did not observe up-regulation of Cav3.1 or Cav3.2 mRNA post-MI, and so if ICaT was up-regulated post-MI, translational, or post-translational mechanisms would have to be involved. We attempted to assess Cav3.1 and Cav3.2 protein expression by western blot, but were unable to do so because of the non-specificity of available antibodies and weak signals at the expected molecular masses. Further evaluation of the adaptive role of Cav3.1-subunits in post-MI remodelling and of the underlying mechanisms would clearly be of interest.

This work was performed in a rodent model and extrapolation to other species, particularly man, should be cautious.
Supplementary material

Supplementary material is available at Cardiovascular Research online.

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