

Arrhythmia and sudden death associated with elevated cardiac chloride channel activity

L. Ye^{a, b, c, †}, W. Zhu^{a, #}, P. H. Backx^{d, e, f}, M. A. Cortez^{g, h}, J. Wu^a, Y.-H. Chow^{a, c, ‡},
C. Mckerlie^{a, c}, A. Wang^a, L.-C. Tsui^{b, i, §}, G.J. Gross^{a, h, j, *}, J. Hu^{a, c, h}

^a Physiology & Experimental Medicine Program, Hospital for Sick Children, Toronto, Canada

^b Genetics and Genomic Biology Program, Hospital for Sick Children, Toronto, Canada

^c Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada

^d Department of Physiology, University of Toronto, Toronto, Canada

^e Department of Medicine, University of Toronto, Toronto, Canada

^f Division of Cardiology, University Health Network, Toronto, Canada

^g Neurosciences and Mental Health Program, Hospital for Sick Children, Toronto, Canada

^h Department of Paediatrics, University of Toronto, Toronto, Canada

ⁱ Department of Medical Genetics and Microbiology, University of Toronto, Toronto, Canada

^j Cardiology Division, Hospital for Sick Children, Toronto, Canada

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Abstract

The identification and analysis of several cationic ion channels and their associated genes have greatly improved our understanding of the molecular and cellular mechanisms of cardiac arrhythmia. Our objective in this study was to examine the involvement of anionic ion channels in cardiac arrhythmia. We used a transgenic mouse model to overexpress the human cystic fibrosis transmembrane conductance regulator (CFTR) gene, which encodes a cAMP-regulated chloride channel. We used RNase protection and *in situ* hybridization assays to determine the level of CFTR expression, and radiotelemetry and *in vivo* electrophysiological study in combination with pharmacological intervention to analyse the cardiac function. Cardiac CFTR overexpression leads to stress-related sudden death in this model. *In vivo* intracardiac electrophysiological studies performed in anaesthetized mice showed no significant differences in baseline conduction parameters including atrial-His bundle (AH) or His bundle-ventricular (HV) conduction intervals, atrioventricular (AV) Wenckebach or 2:1 AV block cycle length and AV nodal functional refractory period. However, following isoproterenol administration, there was marked slowing of conduction parameters, including high-grade AV block in transgenic mice, with non-sustained ventricular tachycardia easily inducible using programmed stimulation or burst pacing. Our sudden death mouse model can be a valuable tool for investigation of the role of chloride channels in arrhythmogenesis and, potentially, for future evaluation of novel anti-arrhythmic therapeutic strategies and pharmacological agents.

Keywords: sudden death • cardiac arrhythmia • chloride channel • cAMP • stress hormone

Introduction

Disturbed myocardial ionic transport mechanisms are critical components of arrhythmia-mediated sudden cardiac death [1]. Genetic studies in cardiac cationic ion channels and their associ-

ated factors have revealed that mutations or changes in expression of several genes enhance arrhythmia susceptibility. These genes include those encoding K⁺ channels and subunits (KCNQ1,

Present addresses:

[†]Cardiovascular Research Institute, University of California, San Francisco, San Francisco, CA, USA

[#]Division of Cardiology, Shanghai Sixth Hospital, Shanghai JiaoTong University School of Medicine, Shanghai, China

[‡]Division of Pulmonary and Critical Care Medicine, University of Washington Harborview Medical Center, Seattle, WA, USA

[§]The University of Hong Kong, Pokfulam, Hong Kong

*Correspondence to: Gil GROSS, Cardiology Division, Labatt Family Heart Centre, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8.
Tel.: (416) 813-7326
Fax: (416) 813-5857
E-mail: gil.gross@sickkids.ca

KCNH2, KCNE1, KCNE2, KCNJ2), Na⁺ or Ca²⁺ channel subunits (SCN5A, RyR2) [2], cardiac-specific transcription factors (Nkx2.5 and HF-1b) [3], and a gap junction channel (connexin40) [4–7]. Dysfunction of these ion transporters, channels and their associated proteins may affect membrane potential and conduction in cardiac myocytes, thus providing a substrate for arrhythmia [2].

Although chloride currents, such as the cAMP protein kinase A dependent currents [8, 9], calcium-activated currents [10–14] and swelling-induced currents [10, 15] modify cardiac electrical activity, their effects on pathogenesis of cardiac arrhythmia are unclear. These chloride currents can contribute to action potential abbreviation as well as resting membrane potential depolarization, thereby increasing susceptibility to arrhythmias [16]. In guinea pig ventricular cells, activation of cAMP protein kinase A dependent chloride currents possibly originating from the cystic fibrosis transmembrane conductance regulator (CFTR) [17] modulates action potential duration and depolarizes the resting membrane potential in a chloride gradient-dependent manner [9]. Interestingly, these chloride channels are not uniformly distributed in all cardiac myocytes [16].

CFTR encodes a cAMP-activated chloride channel, expressed mainly in epithelial cells of the respiratory and digestive tracts [18]. A splice variant of the epithelial CFTR transcript lacking exon 5 was detected in various mammalian heart preparations including rabbit [19], guinea pig [20], human, simian [21, 22] and mouse [23, 24]. In both guinea pig and rabbit ventricle, this current was reported to form a transmural gradient [25] that appears to be developmentally regulated [26]. CFTR was recently shown to play a role in ischemic preconditioning in isolated mouse hearts [27]. However, most cardiac chloride current studies undertaken to date have been restricted to isolated myocytes. Thus, the electrophysiological role of cardiac myocyte chloride currents in the intact heart remains obscure.

Transgenic mouse models can be valuable for studying gene function in hearts [28–33]. We generated transgenic mice expressing the human CFTR gene that encodes a cAMP protein kinase A activated chloride channel. We show that these mice develop stress-related sudden death associated with atrioventricular conduction abnormalities and ventricular arrhythmia.

Materials and methods

Generation of pTREK18iTE CFTR transgenic mice

The plasmid construct used for generating transgenic mice was described previously [34] and transgenic mice were produced as described by Chow *et al.* [35]. A 6715bp *Sap I-Drd I* fragment from pTREK18iTE CFTRtag was used for microinjection and founder mice carrying the transgene were identified by PCR with TRE and cfr3'ss primers [34] and Southern blot analyses. Ten micrograms of genomic DNA from mouse tails were digested with *Pvu II* or *Xba I* and used for Southern blot analyses with the 32P-labelled K18 intron 1 DNA (shown in Fig. 1) as the probe. To rule out an

insertional mutation disrupting a gene or genes essential for normal heart function, we confirmed that in mice with genotype 10–1 or 10–1a the transgene was inserted in mouse chromosome 10 either by fluorescent *in situ* hybridization (FISH) analysis or sequence analysis from isolated flanking regions. F1 animals were obtained by breeding founder mice with wild-type CD-1 or C57Bl6 mice. All studies used heterozygous transgenic mice at the age of 2–13 months.

All animal procedures were approved by the Research Institute of the Hospital for Sick Children Animal Care Committee. The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996).

Riboprobe for RNase protection assay

A 330 bp *Eco RI/Bam HI* fragment of human CFTR cDNA (from nt 3714 to nt 4044) was ligated into pBluescript SK⁺ (Stratagene, La Jolla, CA, USA), and the identity and orientation of the insert fragment were confirmed by sequencing from both the T7 and T3 promoters of the pBluescript vector. The plasmid was linearized by *Xho I* or *Xba I* for preparing antisense and sense probe, respectively. T3 or T7 RNA polymerase was used in *in vitro* transcription.

RNase protection assay

RNase protection assays were performed with RPA IIITM (Ambion[®], Austin, TX, USA) according to the protocol recommended by the supplier, and a β -actin probe was used as an internal control. Briefly, 10 μ g total RNA from mouse tissues was hybridized with RNA probes labelled with [α -³²P]-UTP overnight at 56°C and then treated with RNase T1 for 45 min. A 5% acrylamide gel was used to separate the protected RNA from the degraded unhybridized probe. The protected RNA products were visualized by autoradiography.

Electrocardiogram (ECG) recording by radiotelemetry

To minimize stress during induction and surgery, mice were anaesthetized by isoflurane (Abbott Laboratories, Quebec, Canada) inhalation, then with ketamine (90 μ g/g) and xylazine (10 μ g/g) injected intraperitoneally. A telemetry transmitter (TA10EA-F20; Data Sciences International, St. Paul, MN, USA) was implanted subcutaneously over the scapula, subcutaneous leads placed in the conventional lead II position. Isoproterenol (2.5 μ g/g) (Sigma; St. Louis, MO, USA) was administered through intraperitoneal injection. ECG recording was carried out with the Data Sciences International telemetry system (Data Sciences International, St. Paul, MN, USA). MacLab software (AD Instruments, Inc., Colorado Springs, CO, USA) was used for the analysis.

In vivo electrophysiological study

Fourteen transgenic mice (14–16 week old) and 13 age-matched littermate wild-type mice underwent *in vivo* electrophysiology studies. After anaesthesia, surface ECG with a frequency response of 0.01–100 Hz was obtained and monitored throughout the procedure with body temperature kept constant at 37°C. A 2 French octapolar electrode catheter (NuMed,

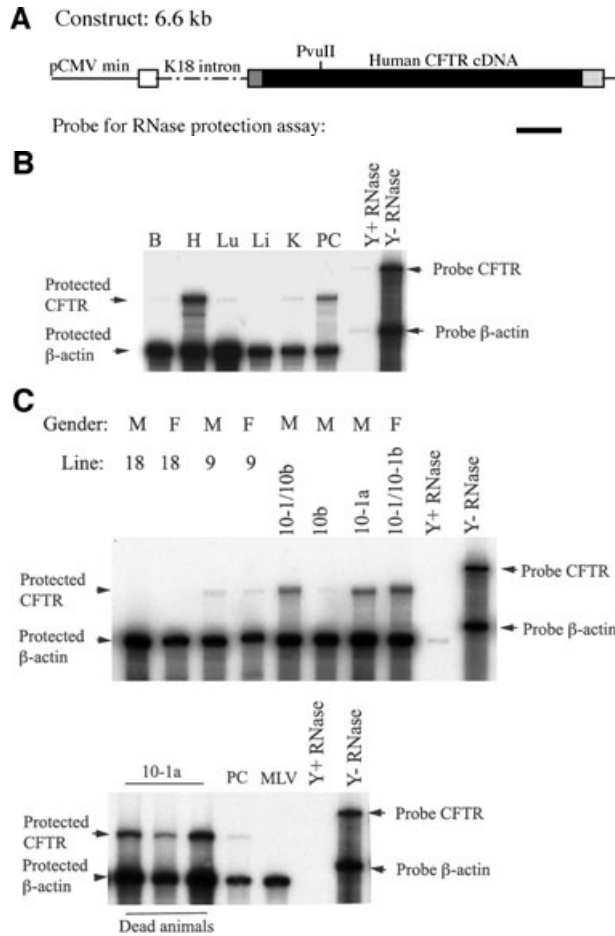


Fig. 1 Generation of CFTR transgenic mice. **(A)** Schematic diagram of a 6.6 kb transgene and strategy for detection of the transgene expression by RNase protection assays. The small open, shaded and stippled boxes represent the TRE element, translational enhancer [33] and VSVG-tag [32], respectively. **(B)** Detection of transgene expression in organs by RNase protection assays. Abbreviations: B, Brain; H, Heart; Lu, Lung; Li, Liver; K, Kidney; PC, positive control (total RNA from a transgenic mouse expressing the human CFTR gene from the K18 promoter); Y + RNase, probes plus yeast RNA treated with RNase T1; Y - RNase, probes plus yeast RNA without addition of RNase T1. **(C)** RNase protection assay analyses of various transgenic lines (top) and dead mice from line 10-1a (bottom). Abbreviations: PC, positive control; MLV, Mouse liver total RNA from the manufacturer as a negative control; Y + RNase and Y - RNase, the same as in Figure 1A.

Inc., Hopkinton, NY, USA) was inserted into the right atrium and across the tricuspid valve through right jugular vein cut-down access. Intracardiac signals with His bundle electrogram were sampled at 2 kHz and filtered at 30 to 500 Hz. The standard pacing protocol was performed in both right atrium and ventricle at double thresholds with 2.0 msec. pulse widths as described previously [36].

Following completion of the baseline electrophysiology study, each mouse received an intraperitoneal injection of isoproterenol (2 ng/g) to examine cardiac conduction under β -adrenoreceptor stimulation. Surface ECG and intracardiac electrograms were continuously recorded with the

Gould Instrument Systems Life Science Suite PONEMAH Physiology Platform (Interfax Systems, Inc., Toronto, Ontario, Canada). Five to 10 min. later, electrophysiological study was repeated for each mouse. PR interval was defined as the interval between the earliest deflection of the P wave and that of the QRS complex. Unpaired Student's t-test was used for comparison between wild-type and transgenic mice groups, whereas paired t-test was used to test the effect of isoproterenol within the same group.

Results

Transgenic mice exhibit cardiac CFTR overexpression

We generated transgenic mice using cytomegalovirus (CMV) minimum promoter to drive human CFTR gene expression (Fig. 1A). Three founder lines (lines 9, 10 and 18) were generated with different copies of the CFTR gene confirmed by Southern blot analysis of genomic DNA isolated from mouse tails (Fig. S1A). Founder line 10 (a female) containing the transgene integrated in different chromosomal locations gave rise to three different types of progeny, named 10-1, 10-1a and 10b (Fig. S1B). Analysis of the genomic structure of 10-1 and 10-1a animals indicated that they differ in the number of tandem repeats of the transgene and the two lines resulted from a single integration followed by rearrangement events (FISH data not shown). The CFTR transgenic mice were born with an expected Mendelian frequency, indicating no embryonic lethality. They grew into adulthood externally indistinguishable from their wild-type littermates. The mRNA expression of the CFTR transgene was particularly high in heart tissue compared to other organs examined (Fig. 1B), indicating preferential CMV promoter expression in myocardium.

Sudden death phenotype correlates with transgenic CFTR expression

To examine human CFTR expression pattern in transgenic mouse hearts, we performed *in situ* hybridization analysis (Fig. S2). CFTR mRNA was detected clearly in the cardiomyocytes of transgenic mice and stronger signal was present in the sudden death group than in the survivor group (data not shown). Sudden death occurred among transgenic mice overexpressing CFTR, typically in presumably catecholamine-charged situations such as fighting and mating, but not among their wild-type littermates. Moreover, there seemed to be a general correlation between spontaneous mortality and degree of CFTR overexpression (Table 1). We obtained ECG tracings of wild-type and transgenic mice using implantable radio telemetry. During most of the monitoring period, the heart rate, QRS duration and PR intervals in the high-level CFTR expression transgenic mice ($n = 5$) were similar to those in wild-type mice ($n = 4$) and C57Bl6 mice ($n = 2$). However, two

Table 1 Mortality rate in hCFTR transgenic mice*

Line		Female	Male	Total	hCFTR expression**
10	10–1	18% (7/40)	62% (18/29)	43% (25/57)	25.95 × ± 9.54***
	10–1a	20% (4/20)	80% (24/30)	56% (28/50)	
	10b	0% (0/7)	0% (0/15)	0% (0/22)	1.00 × ±1.11
9		36% (9/25)	20% (3/15)	30% (12/40)	2.63 × ±1.44
18		0% (0/8)	0% (0/11)	0% (0/19)	1.00 × ±0.88

*All mice are transgene hemizygous (litters are transgenic mice outbred with either C57Bl6 or CD-1 mice. About 5–10 litters of each line was counted).

**The ratio of hCFTR mRNA expression to that of the internal control β -actin in each group $\times 100 \pm$ S.D.

***Indicates the level of expression is significantly higher than other groups. Numbers in parentheses represent spontaneous deaths/total number of animals in group.

high-level transgenic mice exhibited spontaneous irregular heart rhythms and AV dissociation lasting more than 10 min. We observed that pulling the tails of high-level mice provoked ST segment elevation (5 of 5), but no evident ST change appeared in the low level of CFTR expression transgenic mice (0 of 2), wild-type mice (0 of 4) or C57Bl6 mice (0 of 2).

Detection of high levels of transgenic CFTR expression in heart tissue suggests that increased chloride channel activity might contribute to the sudden death phenotype. If this holds true, it is expected that the transgenic mice displaying the sudden death phenotype should have a higher level of CFTR expression. To examine this possibility, we analysed transgenic CFTR expression in three founder lines with RNase protection assays (Fig. 1C). As summarized in Table 1, mice from lines expressing low levels of CFTR, such as lines 18 and 10b, showed no sudden death. Conversely, mice from lines with high levels of CFTR expression (10–1, 10–1a and 9) exhibited high mortality, particularly male mice from lines 10–1 (62%) and 10–1a (80%). We also detected a high level of CFTR expression in the hearts of animals experiencing sudden death (Fig. 1C). These results suggest a correlation between sudden death and CFTR expression in the heart.

Isoproterenol induces AV block and sudden death

To explore whether sudden cardiac death might be linked to a catecholaminergic response to stress, conscious mice were given isoproterenol (2.5 μ g/g) *via* intraperitoneal injection. All five transgenic mice with high levels of hCFTR expression (lines 10–1 and 10–1a, Table 1) died within 5 min. during the recording period, showing repolarization abnormalities, AV conduction block and progressive bradycardia terminating in asystole (Fig. 2). No transgenic mice with a low level of hCFTR expression (line 9, Table 1) or wild-type littermates died after isoproterenol challenge. To

further confirm the effect of isoproterenol on the transgenic mice, we performed *in vivo* intracardiac electrophysiological studies.

In vivo intracardiac electrophysiology studies

As shown in Table 2, before isoproterenol injection transgenic mice and wild-type littermates showed no differences in sinus rhythm cycle lengths (CLs), P-wave duration, PR intervals or QRS duration (Table 2). Intracardiac electrogram recordings of transgenic and wild-type littermates shared similar atrial-His bundle (AH) and His bundle-ventricular (HV) intervals ($P =$ NS, respectively). With overdrive atrial pacing, both transgenic mice and wild-type littermates showed similar AV Wenckebach CL and 2:1 AV conduction CL ($P =$ NS, respectively). With atrial premature pacing, no difference was detected between the two groups in AV node effective refractory period (AVNERP), AV node functional refractory period (AVNFRP) or atrial effective refractory period (AERP) ($P =$ NS, respectively). Ventricular effective refractory period (VERP) was determined by ventricular premature pacing in transgenic mice and wild-type littermates with no statistical difference. No ventricular arrhythmia was induced with overdrive pacing or programmed stimulation in either group of mice under baseline conditions.

Within 5 min. of intraperitoneal isoproterenol injection (2 ng/g), both groups of mice demonstrated similar sinus acceleration. Wild-type littermates additionally showed more rapid AV conduction as well as abbreviated AERP and VERP (Table 2). In contrast, all the transgenic mice responded to isoproterenol β -adrenoreceptor stimulation with paradoxically prolonged PR intervals and widened QRS-T complexes (Fig. 3). Moreover, 7 of 14 CFTR transgenic mice gradually developed severe AV conduction disturbances ranging from transient AV Wenckebach conduction to complete AV block. Although four of the seven mice recovered from transient AV block within 15 min., the other three mice died (Fig. 4A and B). Atrial and ventricular stimulation was completed in 10 of the 11 surviving transgenic mice, demonstrating no residual abnormalities in AV nodal conduction, AERP or VERP following the presumed disappearance of isoproterenol effect.

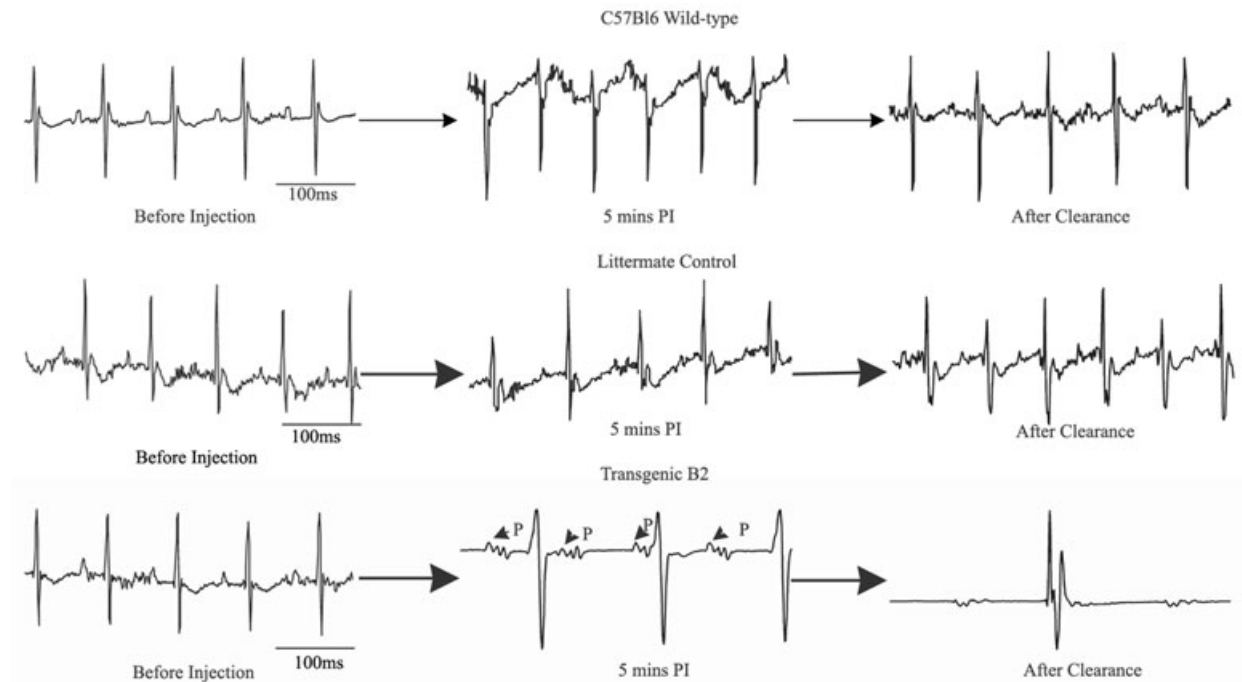


Fig. 2 Representative ECGs recorded from conscious mice with radiotelemetry before and after isoproterenol intraperitoneal injection. C57Bl6 wild-type and littermate control mice were included for comparison. Isoproterenol induced AV block and bradycardia in the transgenic mouse B2. The transgenic mouse, as all other transgenic mice examined, showed bradycardia and died 10 min. after isoproterenol challenge. P indicates P-wave.

Ventricular arrhythmias were induced in 6/10 transgenic mice with programmed ventricular stimulation and in 8/10 transgenic mice using overdrive pacing (Fig. 4C). All these ventricular tachycardias terminated spontaneously. No arrhythmia was induced in wild-type littermates.

Discussion

CFTR overexpression predisposes to sudden cardiac death

In order to assess the potential role of chloride channels in arrhythmogenesis, we used the CMV promoter to overexpress CFTR in the hearts of transgenic mice. Interestingly, mice with a high level of CFTR expression showed a high rate of stress related (*e.g.* fighting or mating) sudden death. This lethal stress effect could be reproduced *in vivo* in transgenic animals with pharmacological β -adrenoreceptor stimulation. Neither spontaneous nor isoproterenol-induced AV block was observed in wild-type littermate controls. These observations are consistent with the known regulation of cardiac chloride channel activity, and CFTR activity in particular, by the β -adrenoreceptor/adenylyl cyclase/protein kinase A system [8, 37, 38].

Phenotype is specific to CFTR overexpression

So far, no spontaneously occurring genes responsible for arrhythmogenesis have been located on mouse chromosome 10. For most mammalian genes, one copy is sufficient, and disruption of one copy of a gene normally does not display any phenotype unless the gene is haploinsufficient. In this rare case, two copies of the gene are required. Because all three independent transgenic lines (9, 10-1 and 10-1a) showed the sudden death phenotype, it is unlikely that disruption of the function of a haploinsufficient gene was the cause of the observed electrophysiological abnormalities.

Correlation of phenotypic abnormalities with cardiac CFTR overexpression pattern

Lack of tissue specificity of CMV promoter can drive CFTR expression throughout the heart. In this transgenic model, we used real-time RT-PCR analysis to demonstrate similar degrees of CFTR expression in atrium and AV node, with preferential expression in ventricular myocardium (Fig. S3). The isoproterenol-induced AV nodal conduction disturbances that we observed can be explained by cAMP dependent, CFTR chloride channel-mediated nodal cell hyperpolarization. Indeed, this effect has previously been observed in mice overexpressing A₃ adenosine receptors

Table 2 ECG and intracardiac electrogram parameters of transgenic and wild-type mice (data expressed as mean ms \pm S.D.; 14 transgenic and 13 wild-type mice were used in this study)

	Pre-Iso transgenic mice	Pre-Iso wild-type mice	Post-Iso transgenic mice	Post-Iso wild-type mice
Sinus Rhythm CL	200 \pm 19	201 \pm 19	135 \pm 4.2 [†]	148 \pm 16 [§]
P duration	14.6 \pm 1.8	16.3 \pm 2.2	22.7 \pm 4.2 [†]	15.2 \pm 1.5
PR interval	45.2 \pm 5.1	44.2 \pm 6.2	53.7 \pm 3.8 [†]	40.7 \pm 6.2 [§]
QRS duration	12.1 \pm 1.7	12.0 \pm 2.1	17.0 \pm 2.3 [†]	11.2 \pm 1.9
AH interval	32.9 \pm 5.0	32.2 \pm 4.7	40.6 \pm 4.7 [†]	29.7 \pm 5.3 [§]
HV interval	12.4 \pm 2.5	11.6 \pm 1.9	13.1 \pm 2.1	11.0 \pm 1.4
AERP	37.1 \pm 13.3	32.3 \pm 7.3	37.4 \pm 9.1	28.5 \pm 6.9 [§]
AV Wenckebach	111.4 \pm 14.6	106.9 \pm 12.5	N/A*	99.2 \pm 7.6 [§]
2:1 AV CL	87.9 \pm 15.3	83.9 \pm 11.9	N/A*	77.7 \pm 8.3 [§]
AVNFRP	108.6 \pm 12.9	101.6 \pm 9.9	N/A*	93.1 \pm 11.1 [§]
AVNERP	85.0 \pm 12.2	78.5 \pm 10.7	N/A*	73.1 \pm 11.1 [§]
VERP	50.1 \pm 16.9	48.3 \pm 9.3	51.4 \pm 9.5	42.2 \pm 7.5 [§]

*Data valid for comparison could not be obtained in the setting of dynamic isoproterenol-induced AV block (see text).

[†] $P < 0.05$ comparison between transgenic mice pre- and post – isoproterenol administration.

[§] $P < 0.05$ comparison between wild-type littermate control pre- and post- isoproterenol administration. $N = 13–14$.

[28]; and purinergic receptors, like β -adrenergic receptors, activate mouse cardiac myocyte CFTR *via* a cAMP-dependent mechanism [39]. The relationship between AV nodal cell hyperpolarization and A-H interval prolongation has been demonstrated by Martynuk *et al.* [40]. Moreover, the mild baseline prolongation of AERP and VERP seen in transgenic mice relative to controls, which was also refractory to isoproterenol-induced shortening (Table 2), lends further credence to the notion that phenotypic effects seen in these animals are largely attributable to CFTR-mediated myocyte hyperpolarization.

Self-limited runs of ventricular tachycardia were inducible, but were not noted to occur spontaneously, in CFTR-overexpressing mice exposed to isoproterenol. This effect was not as dramatic or as lethal as the AV conduction disturbances that we observed, notwithstanding the preferential ventricular expression of the transgene. The chloride reversal potential in cardiac myocytes is thought to be in the -65 to -40 mV range, meaning that catecholamine-mediated CFTR activation would depolarize ventricular myocytes, in contrast to nodal cells which are relatively depolarized at rest and could thus be either depolarized or hyperpolarized by chloride current activation. We speculate that CFTR-mediated depolarization of ventricular myocytes could predispose to the observed ventricular ectopy [41].

Male transgenic mice had a significantly higher spontaneous mortality rate than female mice. This could be due to different levels of stress hormones between males and females, and sex-dependent differences between the degrees of stress associated with fighting and mating. We did not observe any difference

in human CFTR mRNA expression in heart tissues obtained from male and female transgenic mice (Fig. S3). In human studies, women have a significantly lower level of stress hormone compared with men [42]. The sex steroid, oestrogen, might modulate the activity of the hypothalamo–pituitary–adrenal axis and the sympathetic nervous system [43–45]. High levels of stress hormones in male mice would result in cAMP-mediated up-regulation of CFTR channel activity.

Study limitations

Isoproterenol-induced changes in heart rhythm and conduction are assumed to have been attributable to cAMP-mediated CFTR activation. However, our experimental design did not permit exclusion of indirect effects such as vagally mediated conduction changes in response to isoproterenol-induced blood pressure alterations. Nevertheless, even at much higher doses than those employed in our study, isoproterenol given to C57BL6 mice exhibits either no significant acute blood pressure effects [46], or else transient hypotension [47] which, if anything, would be expected to result in reflex tachycardia.

It is well known that CFTR regulates other ion channels [48, 49]. However, our study was not designed to analyse the effects of CFTR expression on other channel activities. Although patch clamp study of chloride currents in isolated AV nodal myocytes would undoubtedly be of interest in this model, we have thus far focused on elucidating effects of cardiac CFTR overexpression *in vivo*.

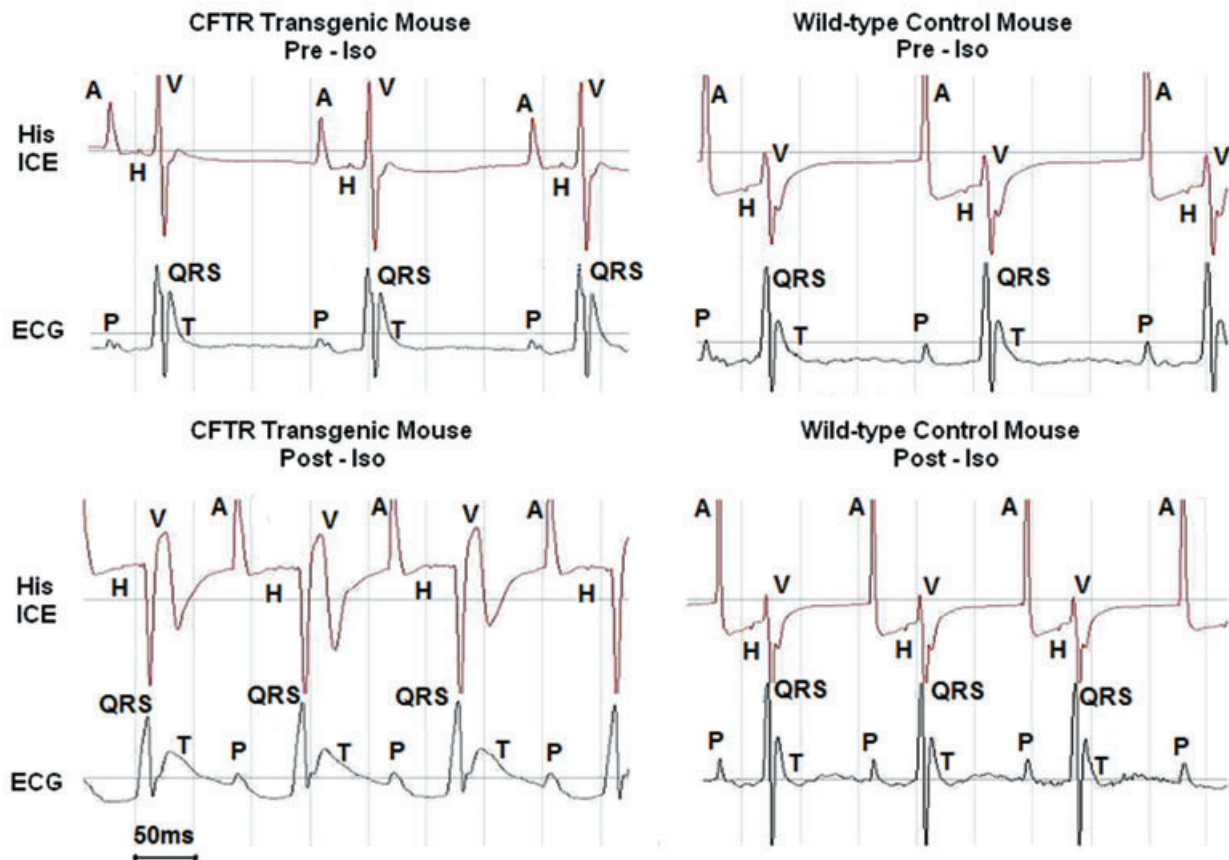


Fig. 3 Sample His bundle electrogram and surface ECG tracings obtained from one anesthetized CFTR transgenic mouse and one wild-type littermate, both pre- and post-isoproterenol administration. At baseline, the PR intervals, QRS duration, QT intervals, AH and HV intervals were similar between transgenic and wild-type mice. However, after isoproterenol injection, the transgenic mice exhibited QT interval prolongation with a giant T wave. A, atrial potential; H, His-bundle deflection; ICE, intracardiac electrogram; V, ventricular potential.

Clinical relevance and implications

In most healthy mammals including human beings, β -adrenergic stimulation accelerates AV nodal conduction. Interestingly, the apparent paradox of isoproterenol-induced AV conduction block that features so prominently in our CFTR overexpression model has been reported only recently in a clinical series of human patients undergoing intracardiac electrophysiology studies [50]. Among 714 patients included in the study, 8 exhibited second degree AV block in response to isoproterenol. The authors speculated that older age or isoproterenol-induced ischemia might play a role, but they were unable to establish a mechanism for this observation.

Our observation of cardiac CFTR overexpression causing stress-associated sudden death in mice suggests the importance of target tissue specificity of CFTR expression in cystic fibrosis gene therapy, because unintended CFTR overexpression in the heart could potentially lead to cardiac arrhythmia. This may not be

a major concern at present because gene delivery and transgene expression are generally inefficient. However, as methods of gene delivery and expression are improved, the cell specificity of therapeutic gene expression should be carefully considered.

Conclusions

Our demonstration of cardiac arrhythmia attributable to excessive CFTR chloride channel activity provides a rationale for considering defects in native cardiac anionic channels as potential substrates in heretofore unexplained cases of cardiac sudden death. The sudden death mouse model described here should provide a valuable tool for investigation of the role of chloride channels in arrhythmogenesis and, potentially, for future evaluation of novel anti-arrhythmic therapeutic strategies and pharmacological agents.

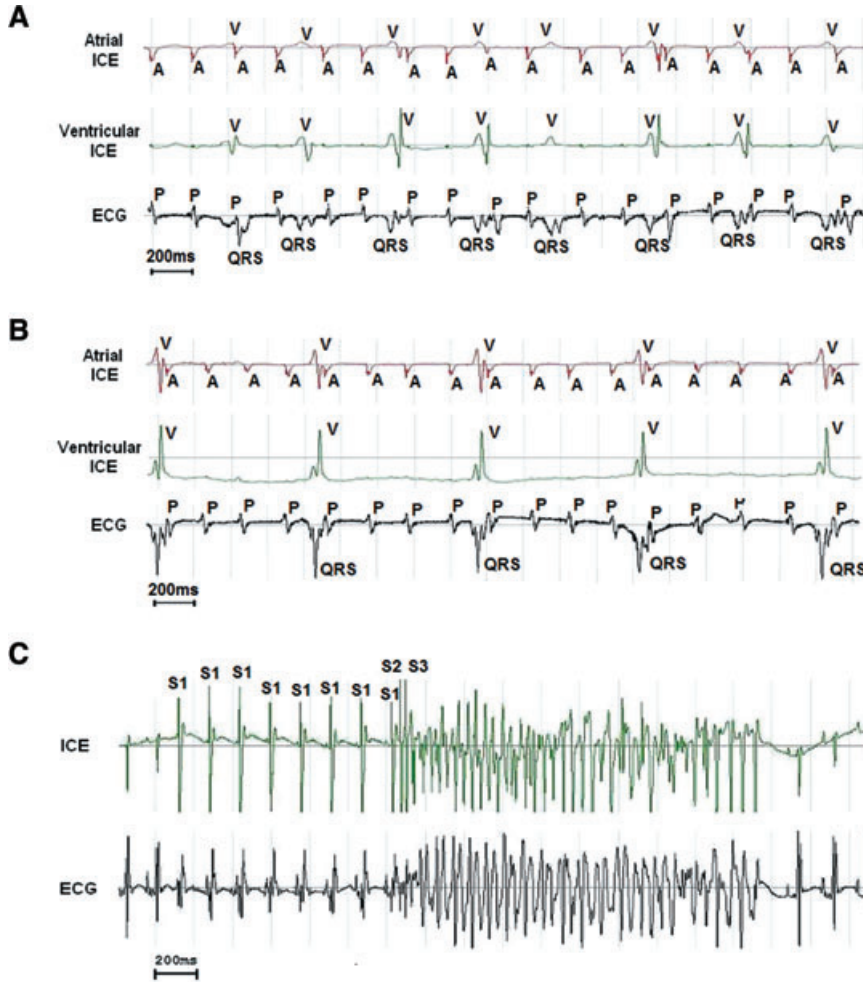


Fig. 4 Intracardiac electrogram and surface ECG tracings of anaesthetized CFTR transgenic mice. **(A)** and **(B)** represent 5 and 10 min after isoproterenol injection, showing progressive high-grade AV conduction block with ventricular rate of 140 beats/min **(A)**, 75 beats/min **(B)**. **(C)** represents an episode of ventricular tachycardia (34 beats with mean CL of 53 msec) induced by programmed stimulation with two extra stimuli after isoproterenol injection.

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Conflict of interest

The authors have certified that there are no conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1 Southern Blot analysis of *CFTR* transgenic mice. **(A)** Identification of transgenic mouse lines. **(B)** Estimation of transgene copy numbers (1.5 to 20 copies) based on the amount of genomic DNA and size of the mouse genome relative the plasmid control used in the analysis. The probe used was K18 intron 1 shown in Fig. 1A. Abbreviations: WT, wild-type; PC, positive control (plasmid DNA).

Fig. S2 Analysis of transgenic expression of the human *CFTR* in mouse heart tissue. **(A)** Expression of the human *CFTR* detected by in situ hybridization with an antisense probe described in 'Methods'. **(B)** A serial section of the same heart hybridized with the sense control RNA. **(C)** A heart section from a nontransgenic littermate probed with the same antisense probe shown in **(A)**. **(D)**, **(E)** and **(F)** Enlarged areas from **(A)**, **(B)** and **(C)**.

Fig. S3 Levels of human (h)CFTR transgene expression in heart tissues of male and female CFTR transgenic mice determined by TaqMan realtime RT-PCR. Total RNA (1 μ g) was reverse transcribed using random hexamers and SuperScript2 reverse tran-

scriptase (Invitrogen) following the manufacturer's protocol. Twenty nanograms of the resulting cDNA were used in real-time PCR performed with a PCR machine from ABI (Prism 7700, Applied Biosystems, Foster City, CA, USA). The sequences of the primers and probe used in the real time PCR are as following: forward primer, CCGAATTCCCGGTCA; reverse primer, GGAGACAACGCTGGCCTTT; and probe, CAAATACTTCCACCATGGAGAG-GTCGCC. A dilution series was used to determine the efficiency of amplification of each primer/probe set, allowing the relative quantification method to be employed (Livak and Schmittgen, Methods

25, 402–408, 2001). 18S was used as a reference gene to normalize the hCFTR expression. Data are expressed as values of $2^{-\Delta Ct} \times 10^3$ versus 18S and are means \pm S.E.M. from six mice in each group. There is no human CFTR transgene expression in transgenic negative littermates.

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