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Nicorandil improves electrical remodelling, leading to the prevention of electrically induced ventricular tachyarrhythmia in a mouse model of desmin-related cardiomyopathy

Naoko Matsushita,*† Masamichi Hirose,* Atsushi Sanbe,‡ Yukiko Kondo,† Yasuyuki Irie† and Eiichi Taira†

**Department of Molecular and Cellular Pharmacology, Iwate Medical University School of Pharmaceutical Sciences, †Department of Pharmacology, Iwate Medical University School of Medicine, and ‡Department of Pharmacotherapeutics, Iwate Medical University School of Pharmaceutical Sciences, Shiwa-gun, Iwate, Japan*

SUMMARY

1. Transgenic (TG) mice overexpressing an arg120gly missense mutation in heat shock protein B5 (*HSPB5*; i.e. R120G TG mice) exhibit desmin-related cardiomyopathy. Recently, the cardioprotective effect of nicorandil has been shown to prolong the survival of R120G TG mice. However, whether the TG mice exhibit ventricular arrhythmias and whether nicorandil can inhibit these arrhythmias remain unknown. In the present study we examined the effects of chronic nicorandil administration on ventricular electrical remodelling and arrhythmias in R120G TG mice.

2. Mice were administered nicorandil (15 mg/kg per day) or vehicle (water) orally from 5 to 30 weeks of age. Electrocardiograms (ECG) and optical action potentials were recorded from R120G TG mouse hearts. In addition, the expression of ventricular connexin 43 and the cardiac Na^+ channel Nav1.5 was examined in TG mice.

3. All ECG parameters tested were prolonged in R120G TG compared with non-transgenic (NTG) mice. Nicorandil improved the prolonged P, PQ and QRS intervals in R120G TG mice. Interestingly, impulse conduction slowing and increases in the expression of total and phosphorylated connexin 43 and Nav1.5 were observed in ventricles from R120G TG compared with NTG mice. Nicorandil improved ventricular impulse conduction slowing and normalized the increased protein expression levels of total and phosphorylated connexin 43, but not of Nav1.5, in R120G TG mouse hearts. Electrical rapid pacing at the ventricle induced ventricular tachyarrhythmias (VT) in six of eight R120G TG mouse hearts, but not in any of the eight nicorandil-treated R120G TG mouse hearts ($P < 0.05$).

4. These findings demonstrate that nicorandil inhibits cardiac electrical remodelling and that the prevention of VT by nicorandil is associated with normalization of connexin 43 expression in this model.

Key words: connexin 43, desmin-related cardiomyopathy, heat shock protein B5, Nav1.5, nicorandil, ventricular tachyarrhythmia.

INTRODUCTION

The anti-anginal agent nicorandil is known as an opener of the ATP-sensitive potassium (K_{ATP}) channel and has a nitrate moiety.[1] Considerable clinical evidence has demonstrated that nicorandil protects the heart against ischaemic injury,[1] improves the recovery of postischaemic contractile dysfunction and can reduce infarct size in several animal models.[2] The Impact Of Nicorandil In Angina (IONA) study, a randomized placebo-controlled study, has shown that nicorandil reduces the incidence of major cardiovascular events in patients with angina pectoris.[1,3] Moreover, recent studies suggest that the cardioprotective effects of nicorandil are mediated by activation of mitochondrial (mito) K_{ATP} channels in myocytes.[2,4]

Desmin mutations in humans cause desmin-related cardiomyopathy, resulting in heart failure, atrial and ventricular arrhythmias and sudden cardiac death. Desmin appears to play a critical role in maintaining the structural integrity of muscle cells and transmitting force generated by contraction by forming a continuous network of filaments that link desmosomes at cell-to-cell adhesion complexes to intracellular components of the contractile apparatus.[5] The R120G missense mutation in heat shock protein B5 (*HSPB5*) can cause desmin-related cardiomyopathy.[6] This disease is a misfolded protein-related disease that can be recapitulated in transgenic (TG) mice by expressing the mutant HSPB5 R120G protein (R120G TG mice) specifically in cardiomyocytes.[7] Hearts from R120G TG mice exhibit perinuclear aggresome formation.[8] In a previous study, we demonstrated that cell viability was dose-dependently recovered in myocytes overexpressing mutant HSPB5 (i.e. R120G TG) following treatment with nicorandil, an opener of K_{ATP} channels and nitric oxide (NO) donor.[9] Nicorandil also inhibited the increase in Bcl-2-associated X (BAX) protein, the decrease in B-cell lymphoma 2 (BCL2), activation of caspase 3 and apoptotic cell death induced by the mutant *HSPB5*.[9] Moreover, nicorandil prolonged the survival of R120G TG mice. On the basis

Correspondence: Dr Masamichi Hirose, Department of Molecular and Cellular Pharmacology, Iwate Medical University School of Pharmaceutical Sciences, Iwate 028-8621, Japan. Email: mhirose@iwate-med.ac.jp

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of these results, we concluded that nicorandil prolonged the survival of R120G TG mice by protecting against mitochondrial impairments.[9] Another study demonstrated the remodelling of gap junctions and slow impulse conduction in a TG mouse model of human desmin-related cardiomyopathy with cardiac-specific expression of a seven amino acid deletion mutation in desmin.[10] However, whether R120G TG mice exhibit cardiac electrical remodelling and whether nicorandil improves the electrical remodelling in these mice remain unknown. In a previous study we demonstrated that nicorandil prevents ventricular tachyarrhythmia induction by improving ventricular electrical remodelling in a mouse model of chronic heart failure.[11] Therefore, we hypothesized that nicorandil improves cardiac electrical remodelling in R120G mice. In the present study, we examined the effects of chronic nicorandil treatment on ventricular electrical remodelling and ventricular tachyarrhythmia (VT) in R120G TG mice.

METHODS

The study protocol was approved by the institutional Animal Experiments Committee of Iwate Medical University and complied with the *Guide for Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH publication 85-23, revised 1996; http://grants.nih.gov/grants/olaw/).

Experimental animals

Mice with cardiac-specific overexpression of mutant HSPB5 containing the R120G mutation, driven by the α-myosin heavy chain promoter, have been described previously.[8] The TG mice were identified by polymerase chain reaction (PCR) analysis of genomic DNA isolated from the tail tips. Non-transgenic (NTG) littermates were always used as controls for comparison. To examine the effects of chronic nicorandil administration on ventricular electrical remodelling and the induction of arrhythmia, R120G TG mice were given nicorandil (15 mg/kg per day; kindly provided by Chugai Pharmaceutical, Tokyo, Japan) or vehicle (water) orally from 5 to 30 weeks of age. All experiments were performed in 30-week-old mice.

Immunohistochemistry

Immunohistochemical analyses were performed as described previously.[8,9,12] Alexa488-conjugated anti-rabbit and Alexa568-conjugated anti-mouse antibodies were purchased from Molecular Probes (Eugene, OR, USA), anti-connexin 43 (Cx43) antibody was from Invitrogen (Camarillo, CA, USA), anti-phosphorylated (p)-Cx43 (Ser^{368}) antibody was from Cell Signaling Technology (Danvers, MA, USA) and the anti-β-catenin antibody was from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Western blotting

Sample preparation for western blotting analysis, gel preparation and electrophoresis were performed as described previously.[8,9,12] Western blot analyses were performed using an anti-GAPDH antibody (Chemicon International, Temecula, CA, USA), an anti-Cx43 antibody (Invitrogen, Camarillo, CA, USA), an anti-p-Cx43 (Ser^{368}) antibody (Cell Signaling Technology) and an anti-Nav1.5 antibody (Aviva Systems Biology, San Diego, CA, USA). Band intensity in the immunoblots was semiquantified using ImageJ software (http://rsbweb.nih.gov/ij/), as described previously.[8,12]

Echocardiography

Mice were anaesthetized with 2% isoflurane and their cardiac function was assessed by echocardiography (Hitachi Aloka Medical, Tokyo, Japan), as described previously.[11] Hearts were viewed at the level of the papillary muscles along the short axis. In M-mode tracings, the average of three consecutive beats was used to measure left ventricular fractional shortening (LVFS).

Electrocardiography

Mice were anaesthetized with 2% isoflurane. Electrocardiogram (ECG) lead II was recorded and filtered (0.1–300 Hz), digitized with 12-bit precision at a sampling rate of 1000 Hz per channel (Microstar Laboratories, Bellevue, WA, USA) and transmitted to a microcomputer before being saved to a CD-ROM.

Langendorff-perfused mouse heart

After being anaesthetized with 2% isoflurane, mice were treated with 3.8 mg/kg, i.v., sodium heparin and hearts were quickly excised and connected to a modified Langendorff apparatus. A polytetrafluoroethylene-coated silver bipolar electrode was used to stimulate the epicardial surface of the anterior left ventricle. A monophasic action potential electrode was placed on the epicardial surface of the posterior left ventricle to record ventricular action potentials. Each preparation was perfused under constant flow conditions with oxygenated (95% oxygen, 5% CO_2) Tyrode's solution (composition (in mmol/L): NaCl 141.0; KCl 5.0; $CaCl_2$ 1.8; $NaHCO_3$ 25.0; $MgSO_4$ 1.0; NaH_2PO_4 1.2; HEPES 5; dextrose 5.0, pH 7.4 at 36 ± 1°C). Perfusion pressure was measured with a pressure transducer (Nihon Kohden, Tokyo, Japan) and maintained within the pressure range 50–60 mmHg by adjusting flow. Preparations were stained with 5 μL voltage-sensitive dye (di-4-ANEPPS; Molecular Probes), dissolved in 0.19 mL ethanol to a concentration of 8 μmol/L. Cardiac rhythm was monitored using three silver disk electrodes fixed to the chamber in positions corresponding to ECG limb leads II.

Optical mapping system

In every experiment, the mapping field was positioned at the anterior left ventricle, including a part of the right ventricle. The optical mapping system used in the present study has been described in detail elsewhere.[13] Briefly, signals recorded from each photodiode and ECG signals were multiplexed and digitized with 12-bit precision at a sampling rate of 3000 Hz per channel (Microstar Laboratories). An optical magnification of ×3 was used, corresponding to a mapping field of 0.6 × 0.6 cm and 0.37 mm spatial resolution between recording pixels.

Experimental protocol

Age-matched NTG, R120G TG and R120G TG + nicorandil-treated (R120G TG + Nico) mice ($n = 8$ in each group) were used. First, ECG lead II was recorded for 10 min in all mice. Second, to measure action potential duration (APD) and conduction velocity (CV), monophasic and optical action potentials were recorded for 10 s from the epicardial surface of the posterior and anterior left ventricles at basic cycle lengths of 200, 150 and 100 msec in isolated hearts. Third, rapid ventricular pacing at a pacing cycle length of 80–100 msec for approximately 5 s from the surface of the anterior left ventricle was performed to induce VT. In the present study, VT was defined as a rapid (cycle length < 100 msec) regular or irregular rhythm persisting for more than 10 beats. Ventricular rapid pacing was performed 10 times to induce VT. Optical action potentials were recorded during the VT from the epicardial surface of the anterior left ventricle for 10 s.

Data analysis

In all anaesthetized mice, P, PR and QRS complex, QT, QTc and RR intervals were measured from ECG lead II. In all monophasic and optical action potentials recorded from Langendorff hearts, depolarization time was defined as the point of the maximum positive derivative in the action potential upstroke (dV/dt_{max}). Depolarization contour maps were computed for the entire mapping field. Repolarization time was defined as the time when repolarization reached a level of 90%. The monophasic APD_{90} was defined as the difference between repolarization time and depolarization time. Mean monophasic APD_{90} was calculated from the average of monophasic APD_{90} from more than three consecutive beats. The upstroke ratio of Phase 0 depolarization was calculated as the ratio of the width of *a* to that of *b* from monophasic and optical action potential. The method of Bayly *et al.*[14] was modified for optically recorded action potential maps for accurate quantification of the direction and magnitude of CV at each recording site. Mean CV was calculated from the average of local CV at more than 100 sites.

All data are given as the mean±SEM. Analysis of variance with Bonferroni's test was used for the analysis of multiple comparisons of data. Fisher's exact test was used to compare the incidence of VT between different conditions. $P < 0.05$ was considered significant.

RESULTS

Cardiac contractile function and heart weight : bodyweight ratio

In the present study, M-mode echocardiograms demonstrated that, compared with NTG mice, R120G TG mice exhibited impaired left ventricular contractility, as demonstrated by the marked reduction in LVFS (Fig. 1a), which is consistent with previous results from TG mice.[8] Nicorandil significantly improved the reduced LVFS in R120G TG mice (Fig. 1a). The heart weight : bodyweight ratio was increased in R120G TG compared with NTG mice, and nicorandil treatment significantly reduced this ratio in R120G TG mice (Table 1).

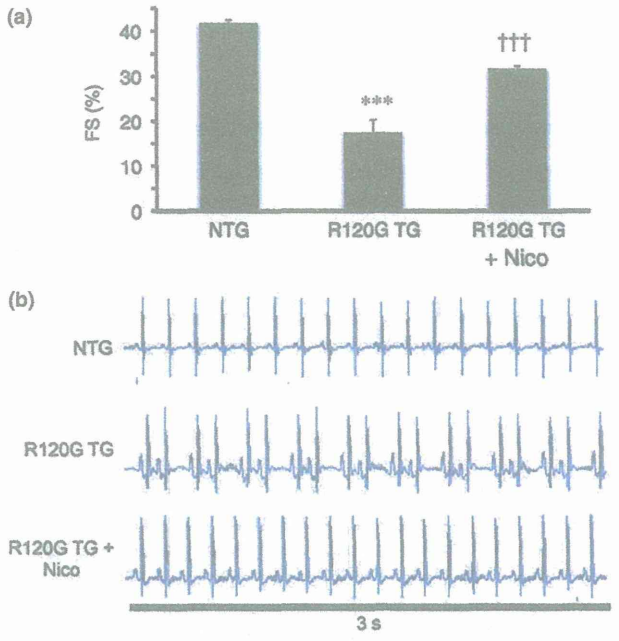

Fig. 1 (a) Left ventricular fractional shortening (FS) in non-transgenic (NTG) mice, mice overexpressing an arg120gly missense mutation in heat shock protein B5 (*HSPB5*; i.e. R120G TG mice) and nicorandil-treated (+ Nico) R120G TG mice. Data are the mean±SEM ($n = 8$ in each group). (b) Representative electrocardiogram lead II recordings from each of the three groups of mice.

Table 1 General parameters and the incidence of ventricular tachyarrhythmia in the three groups

	NTG	R120G TG	R120G TG + Nico
Body weight (g)	31.2 ± 2.5	33.7 ± 1.0	32.8 ± 0.7
Heart weight (mg)	167 ± 5	296 ± 13***	188 ± 15†††
Heart weight : body weight (mg/g)	5.6 ± 0.6	8.8 ± 4.0***	5.7 ± 0.3††
VT induction (Langendorff heart)	0/8	6/8*	0/8†

Data are the mean±SEM ($n = 8$ mice in each group). *$P < 0.05$, ***$P < 0.001$ compared with non-transgenic mice (NTG); †$P < 0.05$, ††$P < 0.01$, †††$P < 0.001$ compared with mice overexpressing an arg120-gly missense mutation in heat shock protein B5 (*HSPB5*; i.e. R120G TG mice).

VT, ventricular tachyarrhythmia; R120G TG + Nico, nicorandil-treated R120G TG mice.

Arrhythmia induction in anaesthetized mice

Figure 1b shows representative ECG recordings from three different groups (NTG, R120G TG, R120G TG + Nico mice) and sinoatrial block or the failure of sinus node function in an R120G TG mouse can be clearly seen. Such sinoatrial block was observed in two of eight R120G TG mice. In contrast, two ECG recordings from NTG and R120G TG + Nico mice showed a P wave and QRS complex with a regular RR interval without any arrhythmia, indicating sinus rhythm. All NTG and R120G TG + Nico mice tested showed sinus rhythm during ECG

recording over a period of 10 min. In addition, ECG recordings for 10 min did not reveal any other arrhythmias in any of the mice in all three groups.

Electrocardiogram parameters in anaesthetized mice

Table 2 gives overall data for electrophysiological parameters in the three groups of mice. All ECG parameters measured, except RR interval and QTc, were prolonged in R120G TG compared with NTG mice. Interestingly, although the QT interval was still prolonged in R120G TG + Nico compared with NTG mice, the P and PR intervals and QRS complex had returned to normal in R120G TG + Nico mice. Moreover, there was no significant difference in QTc among the three groups.

Left ventricular APD and CV

Figure 2a shows representative examples of monophasic action potentials recorded from the epicardial surface of the posterior left ventricle in the three different groups during steady state pacing at a cycle length of 200 msec. Ventricular monophasic APD prolongation was observed in R120G TG and R120G TG + Nico hearts compared with NTG hearts. Mean monophasic APD was significantly prolonged in left ventricles from R120G TG and R120G TG + Nico mice compared with NTG mice at pacing cycle lengths of 200 and 150 msec (Fig. 2b). Interestingly, levels of mean monophasic APD were similar among the three groups at a pacing cycle length of 100 msec. Figure 3a shows representative examples of activation isochrone maps recorded from the epicardial surface of the anterior left ventricle during steady state pacing at cycle lengths of 200, 150 and 100 msec in the three groups. Relative crowding of isochrone lines for R120G TG hearts (Fig. 3b) indicates conduction slowing compared with NTG and R120G TG + Nico hearts (Fig. 3b). In addition, when considering all experiments (Fig. 3b), the mean CV of the left ventricle was significantly slower in R120G TG than NTG hearts, and nicorandil significantly improved the decreased CV in R120G TG hearts.

Induction of VT in Langendorff-perfused heart

Before rapid pacing, spontaneous VT was not observed in any Langendorff-perfused NTG, R120G TG and R120G TG + Nico hearts. Rapid pacing induced VT in six of eight R120G hearts (Table 1). In contrast, rapid pacing did not induce VT in any NTG and R120G TG + Nico hearts (Table 1). Nicorandil significantly reduced the incidence of VT induction by rapid pacing in R120G TG hearts ($P < 0.05$). Figure 4 shows an example of electrically induced VT in a Langendorff-perfused R120G TG heart. A representative ECG and monophasic action potential signal during VT showed rapid repetitive activation at a cycle length of <50 msec without pacing.

Table 2 Electrocardiographic parameters in the three groups

Parameters (msec)	NTG	R120G TG	R120G TG + Nico
P	20 ± 1	34 ± 2***	25 ± 2††
RR	201 ± 20	235 ± 40	203 ± 21
PR	45 ± 5	62 ± 8**	47 ± 8†
QRS	16 ± 1	20 ± 1**	17 ± 1†
QT	34 ± 1	39 ± 2**	37 ± 1*
QTc	77 ± 3	84 ± 4	83 ± 2

Data are the mean±SEM ($n = 8$ mice in each group). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with non-transgenic mice (NTG); †$P < 0.05$, ††$P < 0.01$compared with mice overexpressing an arg120gly missense mutation in heat shock protein B5 (*HSPB5*; i.e. R120G TG mice).

R120G TG + Nico, nicorandil-treated R120G TG mice.

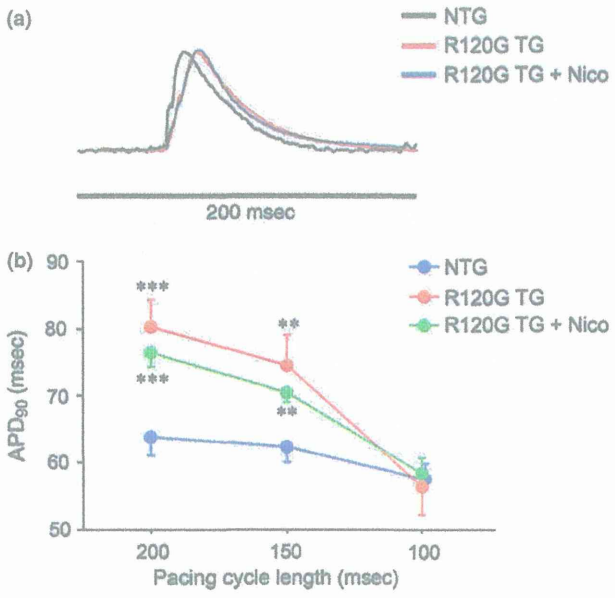

Fig. 2 Monophasic action potential duration (APD) recorded from the epicardial surface of the posterior left ventricle in non-transgenic (NTG) mice, mice overexpressing an arg120gly missense mutation in heat shock protein B5 (*HSPB5*; i.e. R120G TG mice) and nicorandil-treated (+ Nico) R120G TG mice. (a) Representative traces showing monophasic action potentials recorded from the epicardial surface of the posterior left ventricle in the three groups during steady state pacing at a cycle length of 200 msec. (b) Mean monophasic APD_{90} calculated from the average of APD_{90} from more than three consecutive beats. Mean monophasic APD_{90} was significantly prolonged in ventricles from R120G TG compared with NTG mice. Data are the mean±SEM ($n = 8$ in each group). **$P < 0.01$, ***$P < 0.001$ compared with the NTG group.

Connexin 43 expression and spatial distribution pattern

Figure 5a shows the immunolocalization of total Cx43 proteins in the left ventricle from NTG, R120G TG and R120G TG + Nico mice. As can be seen from the immunolabelling distribution patterns of total Cx43 protein, the expression of Cx43 protein was upregulated and linear staining of Cx43 at the lateral cell margins greatly increased in R120G TG compared with NTG hearts. Moreover, Cx43 was heterogeneously stained at intercalated discs of R120G TG compared with NTG mouse ventricles. Interestingly, nicorandil treatment decreased the linear staining of Cx43 at the lateral cell margins in R120G TG hearts. Figure 5c shows Cx43 expression in each group, demonstrating that the anti-Cx43 antibody identified the Cx43 protein and overall expression in left ventricles from mice in each group. The

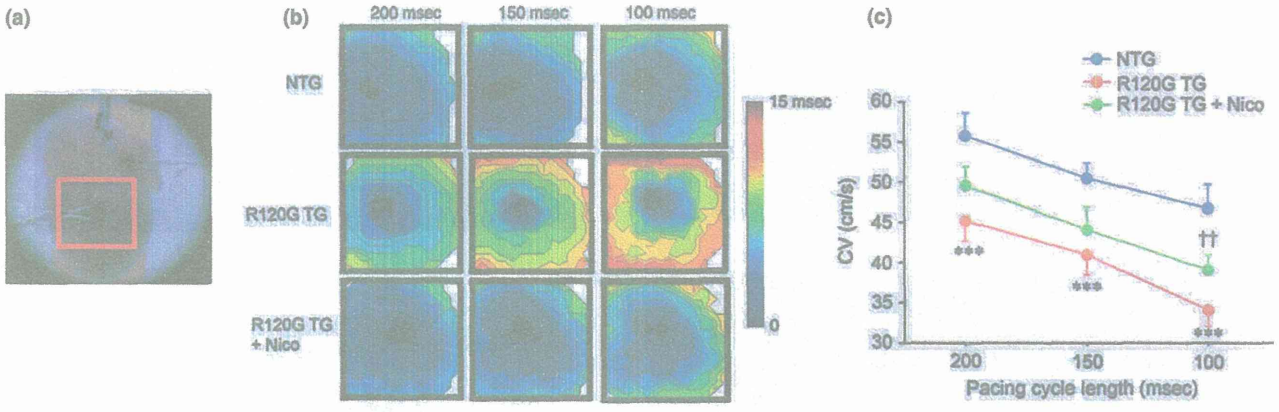

Fig. 3 Impulse conduction velocity (CV) recorded from the epicardial surface of the anterior left ventricle in non-transgenic (NTG) mice, mice overexpressing an arg120gly missense mutation in heat shock protein B5 (*HSPB5*; i.e. R120G TG mice) and nicorandil-treated (+ Nico) R120G TG mice. (a) Photograph of the mapping area (red box) showing the pacing electrode and the anterior left ventricle including part of the right ventricle. (b) Representative activation isochrone maps from each of the three groups during steady state pacing at cycle lengths of 200, 150 and 100 msec. Activation maps are shown with 1 msec isochrones. (c) Mean CV calculated from the local CV at more than 100 sites. Data are the mean±SEM (n = 8 in each group). $^{***}P < 0.001$ compared with the NTG group; $^{\dagger\dagger}P < 0.01$ compared with the R120G TG group.

relative expression of total Cx43 protein was significantly greater in R120G TG than NTG ventricles and nicorandil significantly decreased Cx43 expression to normal levels in R120G TG ventricles (Fig. 5c). Figure 5b shows the immunolocalization of p-Cx43 proteins in left ventricles from NTG, R120G TG and R120G TG + Nico mice. The expression of Cx43 protein was upregulated in R120G TG compared with NTG hearts. Nicorandil treatment decreased the expression of p-Cx43 in R120G TG ventricles (Fig. 5b). Figure 5d,e shows expression levels of p-Cx43 proteins in left ventricular tissue from the three different groups. Similar to total Cx43, the expression of p-Cx43 protein was increased in R120G TG ventricles and nicorandil inhibited this increase in p-Cx43 expression. To determine changes in p-Cx43 and total Cx43 expression, the ratio of p-Cx43 : total Cx43 was calculated (Fig. 5f). There were no significant differences in this ratio among the three different groups.

Upstroke ratio of Phase 0 depolarization and Nav1.5 expression

The upstroke velocity of Phase 0 depolarization depends on Na^+ channel activity and plays an important role in the impulse conduction. Therefore, we examined changes in the upstroke velocity of Phase 0 depolarization using the upstroke ratio of Phase 0 depolarization from monophasic action potentials (Fig. 6a) and the protein expression of Nav1.5 in R120G TG mouse hearts. Figure 6b shows the upstroke ratio of Phase 0 depolarization in the left ventricle in each of the three groups. The upstroke ratio calculated from the monophasic action potential was significantly lower in ventricles from R120G TG than NTG mice, and nicorandil did not improve it (Fig. 6b). In contrast, although the upstroke ratio of Phase 0 depolarization calculated from the optical action potential was significantly lower in ventricles from R120G TG than NTG mice, it was improved by nicorandil treatment (Fig. 6c). Figure 6d,e shows the expression of Nav1.5 proteins in left ventricular tissue from the three different groups. Figure 6d shows a representative example of protein expression in each group and demonstrates that the anti-Nav1.5 antibody

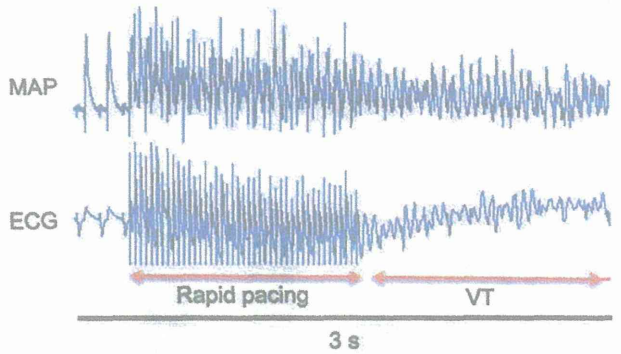

Fig. 4 Example of electrically induced ventricular tachyarrhythmia (VT) in a Langendorff-perfused heart from a mouse overexpressing an arg120-gly missense mutation in heat shock protein B5 (*HSPB5*; i.e. R120G TG group). MAP, monophasic action potential; ECG, electrocardiogram.

identified the Nav1.5 protein. The relative expression levels of Nav1.5 proteins were significantly greater in ventricles from R120G TG than NTG mice, and nicorandil did not improve them in (Fig. 6e).

DISCUSSION

Mean monophasic APD was significantly prolonged in ventricles from R120G TG than NTG mice. Moreover, mean CV for the left ventricle was significantly slower in R120G TG than NTG mice. It is well known that re-entry can induce VT. Re-entry requires a suitable vulnerable substrate. Ventricular remodelling has the potential to increase the likelihood of re-entrant activity through a suitable vulnerable substrate. For example, electrophysiological remodelling, such as refractory period shortening (i.e. APD shortening) and slowing of impulse conduction, can promote re-entry. Re-entry was first defined by Mines in 1914 as a persisting electrical impulse that reactivates an area of previously activated myocardial tissue that is no longer refractory, resulting in a circular movement of activation.[15] The length of such a

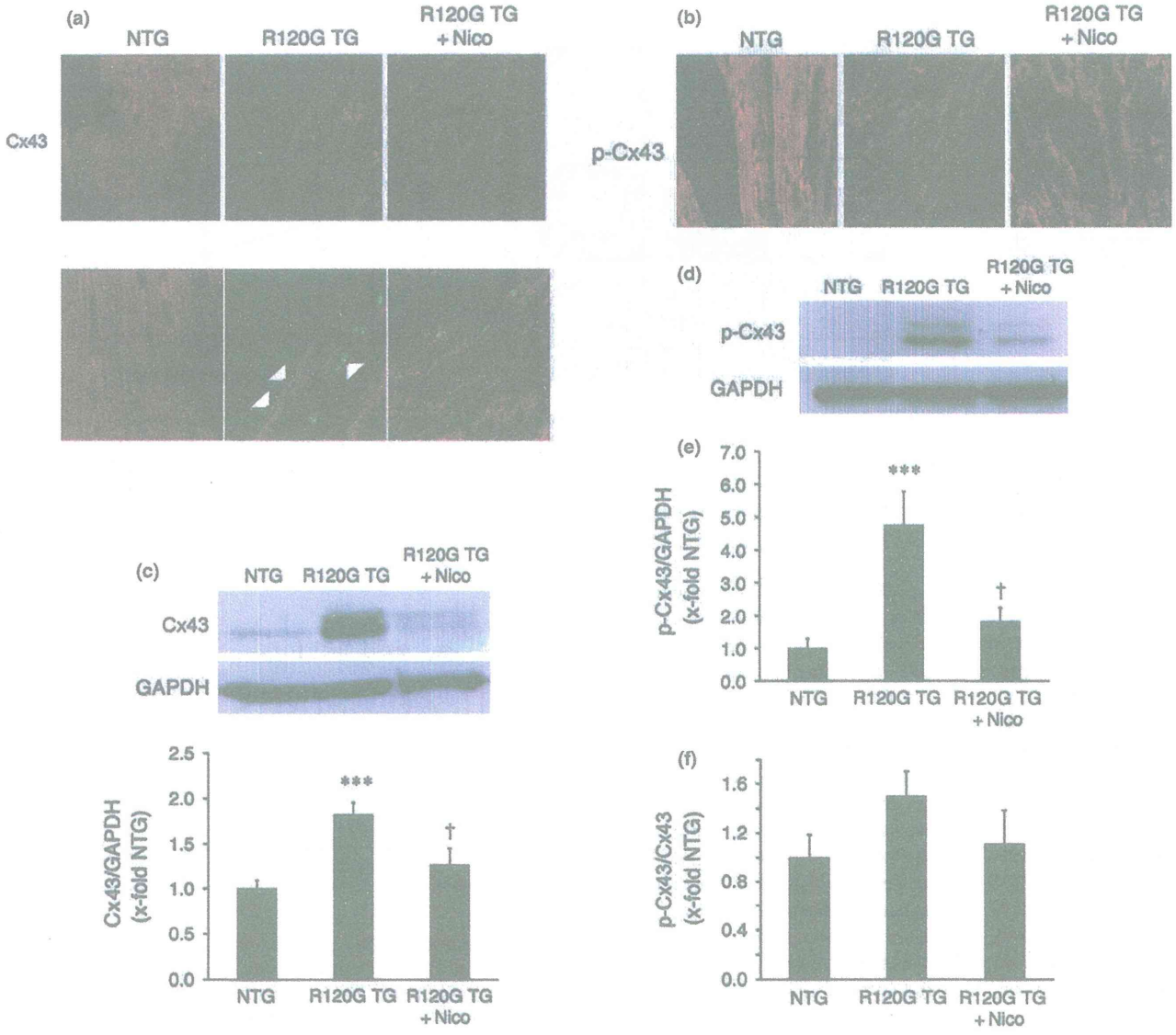

Fig. 5 (a,b) Immunofluorescent confocal images stained for β-catenin (red) and (a) total and (b) phosphorylated (p-) connexin43 (Cx43; green). Longitudinal sections are shown for samples from non-transgenic (NTG) mice, mice overexpressing an arg120gly missense mutation in heat shock protein B5 (*HSPB5*; i.e. R120G TG mice) and nicorandil-treated (+ Nico) R120G TG mice. Arrowheads indicate examples of lateralized staining. (c) Representative immunoblots and summary of results of total Cx43 expression in samples of ventricular tissue from NTG, R120G TG and R120G TG + Nico mice. (d,e) Representative immunoblots (d) and summary of results (e) of p-Cx43 expression in samples from NTG, R120G TG and R120G TG + Nico mice. (f) Ratio of p-Cx43 : total Cx43 protein expression in each of the three groups. In all cases, Cx43 expression was normalized against that of GAPDH expression and is expressed relative to that in the NTG group (set at 1). Data are the mean±SEM from six mice in each group. $^{***}P < 0.001$ compared with the NTG group; $^{\dagger}P < 0.05$ compared with the R120G TG group.

cycle depends on its wavelength, defined by the mathematical product of the refractory period and the CV (plus an excitable gap when present). In the present study, mean monophasic APD was significantly prolonged in ventricles from R120G TG than NTG mice at longer pacing cycle lengths (200 and 150 msec), whereas at a shorter pacing cycle length (100 msec) mean monophasic APD was shorter in ventricles from R120G TG mice, similar to that in the NTG group. Moreover, mean CV for the left ventricle was significantly slower in R120G TG than in NTG mice, suggesting that the electrical remodelling, especially CV slowing, favours the induction of re-entrant activation because of shortening of the wavelength. In fact, electrically induced VT caused re-entry in the present study (see Movie S1, available as Supplementary Material to this paper). Nicorandil did not improve the prolongation of monophasic APD in left ventricles from R120G TG compared with NTG mice, regardless of pacing cycle length, whereas it inhibited decreases in CV in ventricles from R120G TG mice. These results suggest that nicorandil can increase the wavelength of the re-entrant circuit, leading to inhibition of VT. In fact, electrically induced VT was not induced in any of the eight ventricles from nicorandil-treated R120G TG mice in the present study.

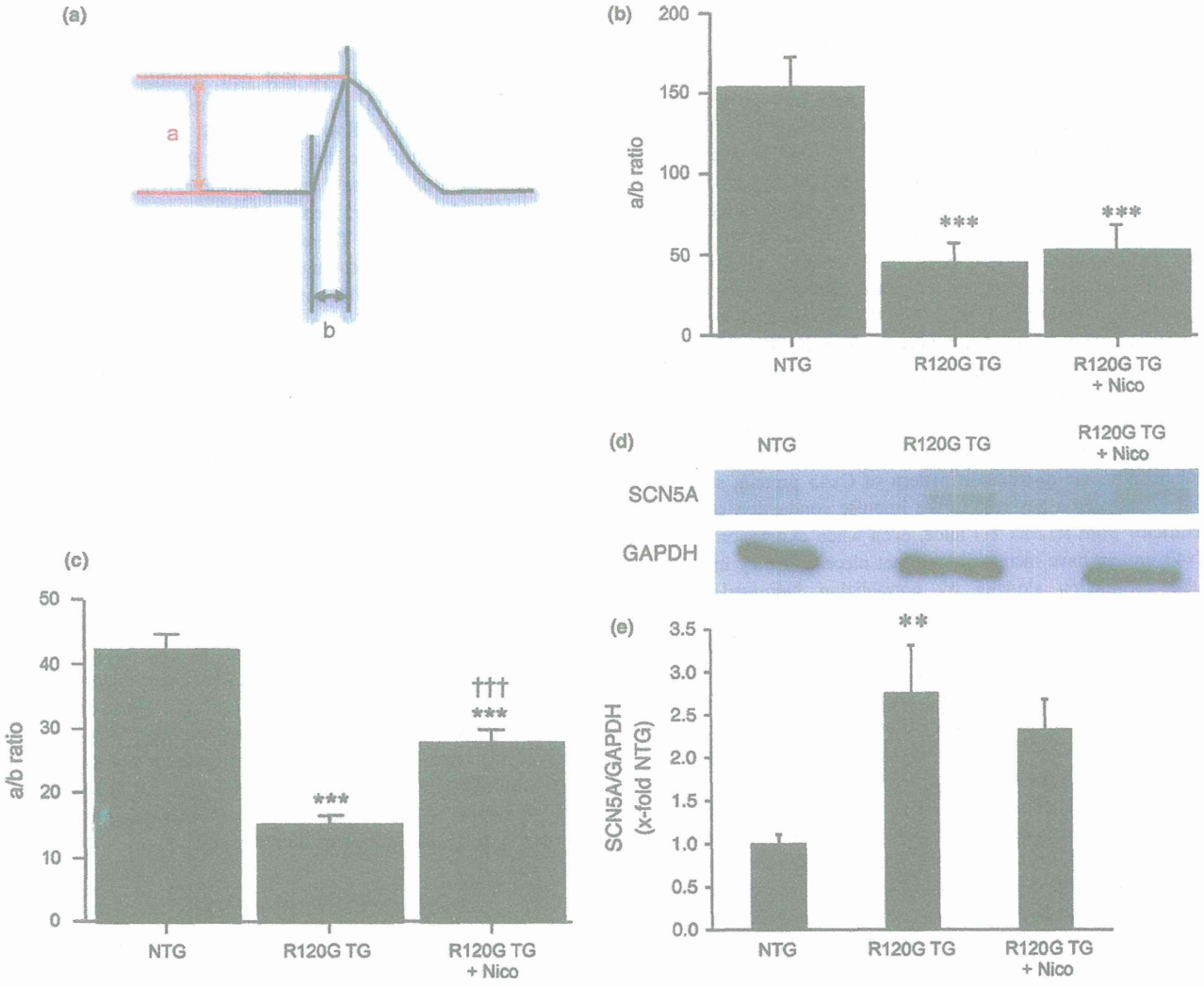

Fig. 6 (a) Method of determining the upstroke ratio of Phase 0 depolarization, calculated as the ratio of the width of *a* to the width of *b* from monophasic and optical action potentials. (b,c) The upstroke ratio of Phase 0 depolarization in the left ventricle calculated from the monophasic action potential (b) and the optical action potential (c) in non-transgenic (NTG) mice, mice overexpressing an arg120gly missense mutation in heat shock protein B5 (*HSPB5*; i.e. R120G TG mice) and nicorandil-treated (+ Nico) R120G TG mice. (d) Representative immunoblots of Nav1.5 (SCN5A) and GAPDH in ventricular tissue from NTG), R120G TG and R120G TG + Nico mice and summary of results of SCN5A expression (normalized against that of GAPDH, with that in the NTG group assigned a value of 1) in the three groups. Data are the mean±SEM (n = 6–8 mice in each group). $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the NTG group; $^{\dagger\dagger\dagger}P < 0.001$ compared with the R120G TG group.

It is generally known that cardiac Cx43 plays key roles in cardiac impulse conduction and that impulse conduction slowing is associated with decreased protein expression levels of Cx43 in hearts.[16] The reasons for the discrepancy in Cx43 expression and impulse conduction in the present study are unknown, but such conflicting data could be explained, in part, by differences in the model of cardiac disease used. In fact, the mouse model of desmin-related cardiomyopathy differs from the model used in the present study, with Gard *et al.*[10] demonstrating that impulse conduction slowing was not associated with decreased Cx43 protein expression. Moreover, a recent study demonstrated that hindlimb unloading resulted in an increased predisposition to ventricular arrhythmia and that arrhythmia induction was associated with increases in left ventricular Cx43 expression.[17] In the present study, we also examined expression levels of p-Cx43 protein because decreases in it are known to cause electrical uncoupling.[18] Contrary to our expectations, p-Cx43 protein expression, like total Cx43 expression, was increased in TG mice. Moreover, the ratio of p-Cx43 : total Cx43 was similar between NTG and R120G TG mice, suggesting that the relative expression of p-Cx43 in total Cx43 is not changed between NTG and TG mice. Interestingly, a recent study suggested that ventricular impulse conduction slowing is associated with increased p-Cx43 expression.[19] These results suggest that factors other than Cx43 protein expression play crucial roles in impulse conduction slowing. Nevertheless, the improvement of conduction slowing was associated with normalized protein expression of total and p-Cx43 in R120G TG mouse hearts following nicorandil treatment, suggesting that increased Cx43 protein expression participates in ventricular impulse conduction slowing in this model.