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Research Article

Down-regulation of sodium current in chronic heart failure: effect of long-term therapy with carvedilol

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Abstract. Evidence has accumulated recently about the importance of alterations in Na^+ channel function and slow myocardial conduction for arrhythmias in the infarcted and failing heart. The present study tested a hypothesis that Na^+ current (I_{Na}/C) density decreases in chronic heart failure (HF) and that Na^+ channel (NaCh) functional density can be restored by long-term therapy with carvedilol, a mixed α- and β-adrenergic blocker. Studies were performed using a canine model of chronic HF produced in dogs by sequential intracoronary embolizations with microspheres. HF developed approximately 3 months after the last embolization (left ventricle, LV, ejection fraction = 28 ± 1%). Ventricular cardiomyocytes (VCs) were isolated enzymatically from LV mid-myocardium, and I_{Na} was measured by whole-cell patch-clamp. The maximum I_{Na}/C was decreased in failing (n = 19) compared to normal (n = 12) hearts (33.1 ± 1.6 vs 48.5 ± 5.1 pA/pF, mean ± SE, $p < 0.001$). The steady-state inactivation and activation of I_{Na} remained unchanged in failing compared to normal hearts. Long-term treatment with carvedilol (1 mg/kg, twice daily for 3 months) normalized I_{Na}/C in dogs with HF. I_{Na}/C in HF dogs (n = 6) treated with carvedilol was higher compared to that of non-treated HF dogs (n = 6) (49.4 ± 0.9 vs 29 ± 4.8 pA/pF, $p < 0.007$). In vitro culture of VCs of failing hearts for 24 h did not restore I_{Na}/C. However, I_{Na}/C was partially restored when VCs were incubated for 24 h with BAPTA-AM, an intracellular Ca^{2+} buffer. Thus, we conclude that experimental chronic HF in dogs results in down-regulation of the functional density of NaCh that can be restored by long-term therapy with carvedilol. The mechanism of NaCh down-regulation in HF may be linked to poor Ca^{2+} handling in this stage of disease.

Key words. Heart failure; sodium channel; patch-clamp; cardiomyocyte; carvedilol.

Sudden cardiac death remains a major public health care problem accounting for nearly 40% of all deaths in patients with heart failure (HF). Ventricular arrhythmias are among the major causes of sudden death in HF patients. Recent studies indicated that alterations in Na^+ channels (NaChs) could contribute to the genesis of cardiac arrhythmias in HF [1]. Down-regulation of NaChs has been found in a canine chronic atrial fibrillation model [2] and in myocytes from the epicardial border zone of infarcted hearts [3]. Since the primary function of NaChs is to initiate cardiac impulse conduction, NaCh alterations can represent a major destabilizing influence within diseased myocardium. Changes in Na current (I_{Na}) can slow myocardial conduction and cause sustained, isolated conduction defects [4] and reentrant arrhythmias [5]. Local or regional electrical instabilities in the myocardial 'substrate' are considered a major risk factor in sudden cardiac deaths [6]. Atherosclerosis, thrombosis, and infarction resulting from coronary artery disease represent the largest predisposing factor for lethal arrhythmias [for a review see ref. 7]. In addition to arrhythmias, another important aspect of the slow trans-myocardial conduction is

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poor hemodynamic performance of the failing heart. In fact, patients with HF frequently manifest intraventricular conduction delays, which can be treated by cardiac resynchronization therapy [8].

The present study tested the following hypotheses: (i) the functional density of NaChs is down-regulated in left ventricular (LV) myocardium of dogs with chronic HF, (ii) decreased functional density of NaChs in failing hearts can be restored by long-term therapy with carvedilol, a mixed α- and β-adrenergic blocker, and (iii) the decreased functional density of NaChs in HF dogs is sustained and can be restored in vitro by influencing intracellular Ca^{2+} homeostasis. Carvedilol was employed in this study because of its beneficial effects in patients with chronic HF [9, 10] and its possible regulatory role on voltage-gated NaCh expression [11].

Taking into account known difficulties in human studies, a reproducible canine chronic HF model that manifests marked and sustained depression of ventricular function, dense ventricular ectopy, and sudden death in ~13% of animals [12, 13] was employed in the study. This canine chronic HF model simulates human coronary artery disease. Multiple diffused infarctions were produced by coronary embolizations with microspheres. Chronic HF developed approximately 3 months after the last embolization. To avoid misinterpretations due to transmural regional differences in NaCh expression [14], voltage-clamp studies were performed in LV mid-myocardial cardiomyocytes (VCs).

Materials and methods

Canine chronic HF model

The dog model of chronic HF has been previously described in detail [12]. Healthy mongrel dogs, weighing between 24 and 31 kg, underwent multiple sequential coronary microembolizations, to produce HF. Embolizations were performed 1–3 weeks apart and were discontinued when LV ejection fraction, determined angiographically, was ≤35%. At the time of harvesting the heart (~3 months after the last microembolization) the LV ejection fraction was 28 ± 1%. LV tissue specimens were obtained from a total of 12 normal and 31 HF dogs, including those randomized for the carvedilol study (see below). This study met the 'Ethical principles and guidelines for scientific experiments on animals' of the Swiss Academy of Medical Sciences and was approved by the Henry Ford Health System Human Rights Committee Institutional Review Board.

Design of the carvedilol study

The carvedilol study population represents a sub-study of a larger trial. To test the effect of chronic therapy with carvedilol, LV dysfunction and failure were produced in 12 dogs. Three weeks after the last embolization, dogs were randomized to 3 months of oral therapy with carvedilol (1 mg/kg, twice daily, n = 6), or no treatment (control, n = 6). After the therapy, I_{Na} was measured in VCs of both groups.

VC isolation

Cells were enzymatically isolated from slices of the apical third of the LV mid-myocardium as previously described [15]. The yield of viable, Ca^{2+}-tolerant, rod-shaped VCs isolated from both normal and failed hearts varied from 20 to 80%.

Culture of VCs

To determine the effect of long-term intracellular Ca^{2+} buffering on NaCh expression, VCs were cultured as previously described [16]. The culturing serum-free medium M199 (Earl's salts without L-glutamine buffered with 25 mM N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid] (HEPES) and bicarbonate) was supplemented with carnitine 2 mmol/l, creatine 5 mmol/l, taurine 5 mmol/l, albumin 2 g/l (bovine, fraction V), insulin 0.1 µmol/l, penicillin 100 U/ml, streptomycin 100 µg/ml, and gentamycin 25 µg/ml. The cells were incubated at 37 °C in a humidified atmosphere of 5% carbon dioxide. In our culture conditions, after 24 h of incubation, VCs showed moderate changes in cell shape and morphology mainly resulting in slightly rounded edges of the cultured cells (fig. 1).

Patch-clamp measurements

I_{Na} was recorded by a whole-cell patch-clamp technique as we previously reported [17]. The pipette solution contained (in mmol/l): CsCl 133, NaCl 5, ethylene glycol-bis(beta-aminoethyl ether)N,N,N′,N′-tetraacetic acid (EGTA) 10, MgATP 5, tetraethylammonium 20, HEPES 5 (pH 7.3 with CsOH). The patch pipette tip resistance was 0.5–0.8 MΩ. Whole-cell currents were measured at room temperatures of 22–24 °C in a symmetric Na^+ (5/5 mM) conditions, with the bath solution containing (in mmol/l): NaCl 5, CsCl 133, $MgCl_2$ 2, $CaCl_2$ 1.8, nifedipine 0.002, HEPES 5 (pH 7.3 with CsOH). Whole-cell I_{Na} was obtained by subtracting the traces elicited with the comparable voltage steps containing no I_{Na} from raw current traces. The peak of I_{Na} ranged from 2 to 10 nA. The quality of the voltage clamp was controlled in each VC by estimating deviation (V_{dev}) from voltage command as previously described [17]. Satisfactory voltage control was assumed if $V_{dev} < 2$ mV, and only those cells which met this criterion were included in the study.

Experimental protocols and data analysis

Data were analyzed using the pClamp 8 software (Axon Instruments). The experimental protocols and data analysis were similar to those we reported previously [17]. The

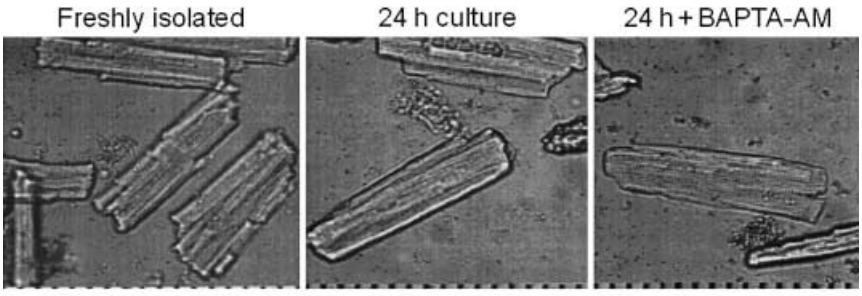

Figure 1. Examples of failed canine VCs isolated from mid-myocardium of left ventricle: freshly isolated (left panel), after 24 h in cell culture (middle panel), and treated for 24 h with a cell-permeable Ca^{2+} buffer (BAPTA-AM, 20 mmol/l, right panel). Cells were attached to a laminin-covered surface. Calibration ticks, 10 µm; objective magnification, ×25.

steady-state availability parameters ($V_{1/2A}$, the mid-point and k_A, the slope of the relationship) were measured by a standard double-pulse protocol with the 2-s-duration pre-pulse (V_p) ranging from –140 mV to –40 mV. The data points of I_{Na} normalized to maximum I_{Na} measured at –140 mV pre-pulse were fitted to a Boltzmann function $A(V_p)$:

$$A(V_p) = 1/(1 + \exp((V_p - V_{1/2A})/k_A)) \quad (1)$$

The steady-state activation parameters were determined from the I_{Na}-voltage relationships by fitting data points of the normalized current with the function:

$$I_{Na}(V_t)/C_m = G^x_{max} (V_t - V_r)/\{1 + \exp[(V_{1/2G} - V_t)/k_G]\} \quad (2)$$

Where G_{max} is a normalized maximum Na^+ conductance, V_r is a reversal potential, $V_{1/2G}$ and k_G are the mid-point and the slope of the respective Boltzmann function underlying the steady-state NaCh activation. The data points I_{Na}/C_m were fitted by Clampfit 8 software using an option 'Custom function' with four independent parameters corresponding to G_{max}, V_r, $V_{1/2G}$, and k_G. The peak current (I_{Na})-voltage relationship was determined in each cell with a series of depolarization pulses of 50-ms duration applied to different testing voltages (V_t) with a rate of 0.5 Hz from a holding potential of –140 mV. I_{Na} density was determined from these I_{Na}-voltage relations as the maximum peak I_{Na} (V_t= –30 mV) normalized to membrane electric capacitance, C_m, measured by a voltage ramp pulse with a slope (dV/dt) of –10 V/s from –80 to –100 mV. The C_m values were calculated according to $C_m = (I_{ramp} - I_{ss})/(dV/dt)$, where I_{ss} is the steady-state level of membrane current at –100 mV and I_{ramp} is membrane current at the end of the ramp pulse.

Chemicals

All chemicals and cell culture components including 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetra-acetic acid tetraacetoxymethyl ester (BAPTA-AM) were purchased from Sigma (St. Louis, M.). Carvedilol was obtained from Daiichi (Japan).

Statistics

If not specifically stated, all measurements are reported as the mean ± SE with 'n' representing the number of hearts. Comparison between mean values was performed with an unpaired Student's t test. A level of $p < 0.01$ was considered significant.

Results

We performed patch-clamp experiments on both freshly isolated and cultured VCs (fig. 1). Freshly isolated VCs were used to study the effects of chronic HF and long-term therapy with carvedilol on I_{Na}. VCs isolated from dogs with HF were incubated in vitro to test whether down-regulation of I_{Na} in those cells was stable over time when they were removed from their 'pathological' environment. We also tested whether I_{Na} could be restored in vitro by the mechanisms related to intracellular Ca^{2+} homeostasis.

I_{Na} density is significantly decreased in chronic HF

The whole-cell Na^+ currents were compared using VCs isolated from hearts of two groups of animals of similar age and weight: (i) a group of 12 normal dogs and (ii) a group of 19 dogs with a reduced LV ejection fraction and HF. To minimize effects of the shifts known for steady-state availability and activation [17], the measurements of I_{Na} were performed approximately 25 min after membrane cell rupture. The currents were normalized to cell membrane capacitance, which on average was significantly higher in VCs isolated from failing hearts (FVCs) compared to those isolated from normal hearts (NVCs): 202 ± 12 pF (n = 106) versus 140 ± 10 pF (n = 52), respectively. I_{Na} density in FVCs was decreased compared to NVCs (48.5 ± 5.1 pA/pF vs 33.1 ± 1.6 pA/pF, $p < 0.001$; fig. 2). The currents were of Na^+ origin, as they could be completely blocked by 25 µmol/l of tetrodotoxin, a specific blocker of NaChs (not shown). The currents reversed at around 0 mV, in line with the symmetrical Na^+ concentration used in our experiments.

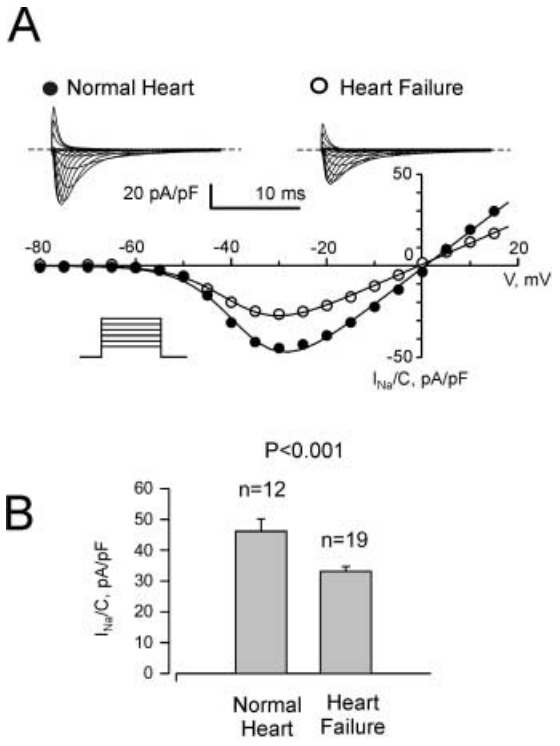

Figure 2. Down-regulation of Na^+ current density (I_{Na}/C) in mid-myocardium ventricular myocytes in infarction-originating heart failure. (*A*) Representative examples of I_{Na} density-voltage relationships in normal and failing myocardium. The solid lines show theoretical curves fitted in accordance with equation 2 (see methods). The upper graphs show respective families of I_{Na} current traces along with a schematic representation of the voltage-clamp protocol. (*B*) Average data for maximum I_{Na} density measured in 12 normal and 19 failing canine hearts (52 and 106 cardiomyocytes, respectively). Shown are the mean ± SE for n, number of hearts used in the study.

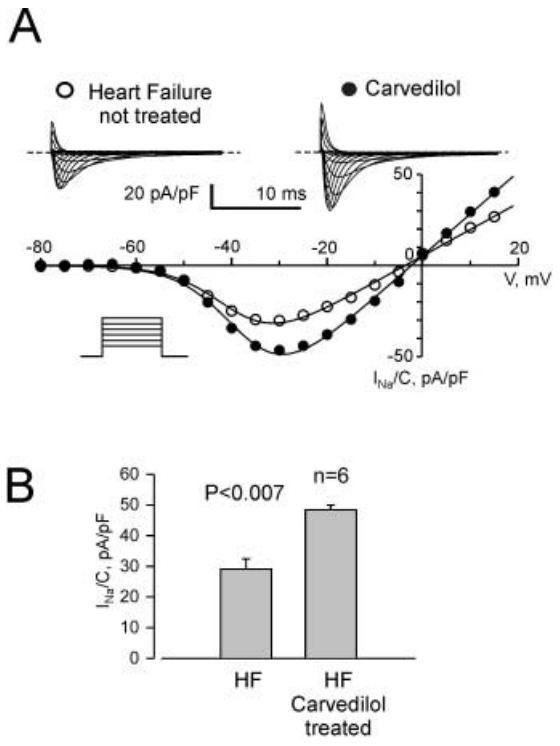

Figure 3. Recovery of Na^+ current density (I_{Na}/C) in mid-myocardium ventricular myocytes in infarction-originating heart failure by chronic therapy with carvedilol. Shown are data of a separate randomized study with chronic therapy of HF dogs with carvedilol. (*A*) Representative examples of I_{Na} density-voltage relationships in cardiomyoctes isolated from carvedilol-treated and carvedilol-untreated canine failing myocardium. The solid lines show theoretical curves fitted in accordance with equation 2 (see methods). The upper graphs show respective families of I_{Na} current traces along with a schematic representation of the voltage-clamp protocol. (*B*) Average data for maximum I_{Na}/C measured in six carvedilol-treated and six carvedilol-untreated failing canine hearts (55 and 32 cells, respectively). Chronic treatment with carvedilol was performed with 1 mg/kg, twice daily for 3 months. Shown are the mean ± SE for n, number of hearts used in the study.

I_{Na} density is recovered by long-term monotherapy with carvedilol

Once we found down-regulation of I_{Na} density in FVCs, we tested whether the density change could be recovered both in vivo and in vitro. To test whether Ina density could be recovered in vivo, we treated HF dogs for 3 months with carvedilol (see Materials and methods for details). I_{Na} density in FVCs from dogs (n = 6) undergoing chronic therapy with carvedilol was similar to that of NVCs. At the same time, the I_{Na} density was significantly higher compared to that of untreated HF dogs (n = 6) within the same randomized group of animals (49.4 ± 0.9 pA/pF vs 29 ± 4.8 pA/pF, $p < 0.007$, fig. 3).

In vitro culture of HF cardiomyocytes does not recover I_{Na} density

Once we found down-regulation of I_{Na} density in FVCs, an important question was whether this change was stable over time or just a reflection of altered external environmental factors such as extracellular matrix, cell-to-cell contacts, and/or soluble factors, e. g., cardiac hormones and neuropeptides. If the latter were the case, we would expect that the density might recover over time when the cells are removed from their 'pathological' environment. To test the importance of the cell environment for I_{Na} down-regulation, we cultivated FVCs in vitro for up to 72 h in medium containing only standard essential cell culture components. We also tested spontaneous I_{Na} density changes in vitro in NVCs. We found that I_{Na} density of both NVCs and FVCs was stable (although there was a slight ~3 %/24 h decrease) during cultivation (fig. 4A). This result indicates that the decrease in I_{Na} density in FVCs is not just a reflection of the extracellular environment but is an intrinsic property of VCs in this disease state.

Long-term intracellular Ca^{2+} buffering results in a partial recovery of I_{Na}

Once we had found that I_{Na} density decrease in FVCs was stable, the next important question was whether the den-

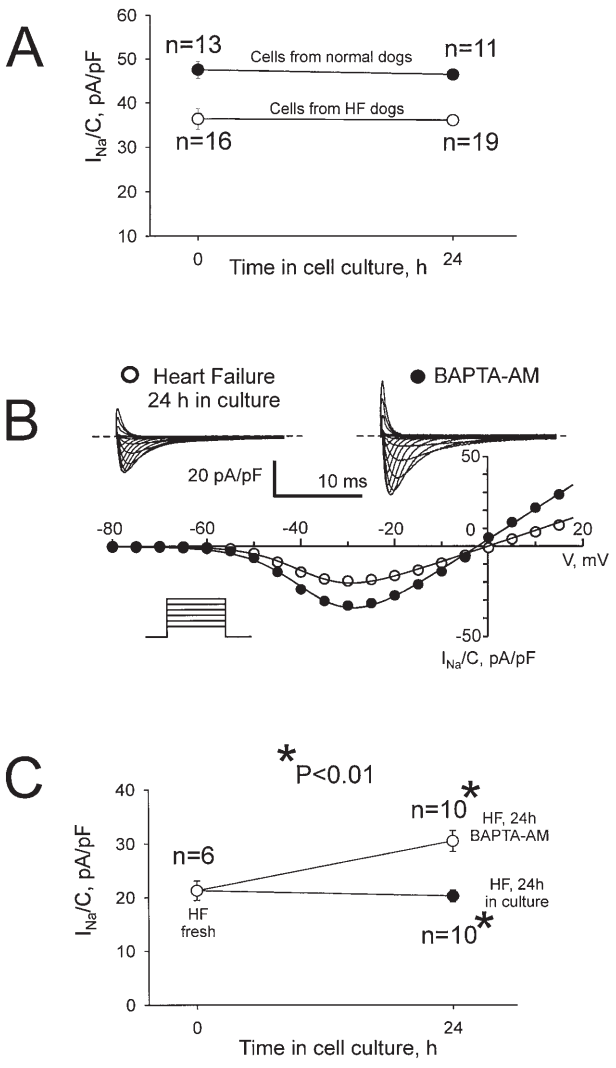

Figure 4. Partial recovery of I_{Na} density (I_{Na}/C) in cardiomyocytes isolated from failed canine hearts by long-term intracellular Ca^{2+} buffering with BAPTA-AM (20 mmol/l) in cell culture (24 h). (*A*) I_{Na}/C of cardiomyocytes of both normal (closed circles) and failing (open circles) hearts was stable during a 24-h cultivation period. (*B*) Representative examples of I_{Na} density-voltage relationships in cardiomyocytes incubated with and without BAPTA-AM. The solid lines are theoretical curves fitted in accordance with equation 2 (see methods). The upper graphs show respective families of I_{Na} current traces along with a schematic representation of the voltage clamp protocol. (*C*) Average data for maximum I_{Na} density. Shown are the mean ± SE for n, number of cardiomyocytes measured. Cardiomyocytes shown here were isolated from two normal and two failing dog hearts.

sity could still be recovered in vitro by a conventional mechanism for NaCh regulation in cardiomyocytes. As previously reported by Chiamvimonvat et al. [18], intracellular Ca^{2+} in cultured neonatal rat cardiac myocytes regulates the number of functional NaChs. We followed their experimental protocol and tested whether I_{Na} density in FVCs could be increased by modulating intracellular Ca^{2+}. Accordingly, isolated failed cardiomyocytes were incubated with BAPTA-AM (20 mmol/l in 0.04% DMSO final), a cell-permeable version of BAPTA, a well-known strong Ca^{2+} buffer. Cell incubation with BAPTA-AM began on the first day of cell culture and lasted for 24 h. The morphology of BAPTA-AM-treated VC was similar to that of untreated cells (fig. 1). In VCs incubated for 24 h with BAPTA-AM, I_{Na} density was significantly higher than that in cultured VCs under identical conditions but without BAPTA-AM (31.4 ± 2.9 pA/pF, n = 6, vs 20.9 ± 2.1 pA/pF, n = 10, $p < 0.027$; respectively; fig. 4B, C).

I_{Na} density changes were not related to steady-state activation and availability

To ensure that the observed changes in I_{Na} density were not related to changes in voltage dependence of steady-state activation and availability, we measured these parameters in each set of experiments. The parameters remained almost unchanged throughout the study (table 1). Accordingly, both NVCs and FVCs exhibited identical I_{Na} activation thresholds, which occurred in the range from –60 mV to –55 mV. For both cell types, the currents reached a maximum peak density at voltages ranging between –30 mV and –25 mV, similar to findings of others in normal canine cardiomyocytes under similar conditions [3]. The parameters of steady-state activation and availability also remained almost unchanged in VCs of carvedidlol-treated dogs versus untreated HF dogs (table 1) as well as in all sets of in vitro VC-culturing experiments (data not shown).

Discussion

For the first time, we demonstrated down-regulation of functional expression of NaChs in ventricular cardiomyocytes in chronic HF. This study also demonstrated that long-term therapy with the mixed α- and β-adrenergic blocker carvedilol can recover functional NaCh density in chronic HF. Our results suggest that NaCh down-regulation, in part, is due to poor Ca^{2+} handling by cardiomyocytes of failing hearts.

Possible clinical relevance

We believe these data have clinical relevance and, importantly, extend prior observations. First, the I_{Na} down-regulation found in the present study may provide a new mechanism for the development of ventricular arrhythmias in infarction-originated HF such as that due to coronary artery disease. This mechanism can act in concert with the already established mechanism via down-regulation of K^+ channels [19] in HF. Indeed, together with geometric non-uniformities (structural alterations) within the healed infarction tissue (including fibrosis or myocardial scarring) and decreased cell-to-cell coupling in HF [20], the decline of functional NaCh density reported in the present study can contribute to the incidence of unidirec-

Table 1. Voltage dependence of steady-state availability ($V_{1/2A}$ and k_A) and activation ($V_{1/2m}$ and k_m) for Na^+ current remained unchanged in all preparation of cardiomyocytes used in the study.

Preparation	$V_{1/2A}$ (mV)	k_A (mV)	$V_{1/2G}$ (mV)	k_G (mV)
Normal heart	−81.5 ± 1	5.02 ± 0.07	−36.3 ± 1.0	6.2 ± 0.07
Failing heart	−82.3 ± 1.5	5.08 ± 0.21	−37.2 ± 1.5	6.3 ± 0.2
Failing hearts treated with carvedilol	−80.3 ± 1.3	5.10 ± 0.07	−36.2 ± 1.0	6.4 ± 0.1
Untreated failing hearts	−80.5 ± 0.7	5.11 ± 0.06	−35.4 ± 0.7	6.3 ± 0.06

Parameters were obtained from Na^+ current recordings using the protocols and fitting function described in Material and methods (equations 1 and 2). Data for 'Normal heart' and 'Failing heart' were obtained from 12 hearts (52 cells) and 19 hearts (106 cells), respectively. Data for hearts shown as 'Untreated failing hearts' (6 hearts, 32 cells) were obtained from dogs of the same randomized carvedilol study shown as 'Failing hearts treated with carvedilol' (6 hearts, 55 cells). Chronic treatment with carvedilol was performed with 1 mg/kg, twice daily for 3 months. Shown are the mean ± SE calculated for n number of hearts.

tional block, slow down impulse propagation, and facilitate re-entrant arrthythmias (e.g., around scar tissue). Similar changes in I_{Na} have recently been shown to slow myocardial conduction and cause sustained, isolated conduction defects [4].

Second, this mechanism can explain the pro-arrhythmic effect of class I antiarrhythmic drugs, as further blockade of down-regulated NaChs can make excitation propagation even worse. This idea is consistent with results of clinical trials (e.g., CAST), which indicated that class I antiarrhythmic drugs increase mortality in HF patients. The results of the present study and the ease of pro-arrhythmic manifestations specific to class I antiarrhythmic drugs in HF patients indicate a possible involvement of NaChs in arrhythmias in this disease state. Alternatively, amiodarone known by its preferential blockade of the late Na^+ current (I_{NaL}) compared to the peak transient I_{Na} [21] has a low proarrhythmic profile and represents, currently, the most effective antiarrhythmic drug, widely used in clinics.

Third, chronic therapy with β-blockers significantly decreases the risk of sudden death in HF patients [22]. The present study shows that long-term therapy with carvedilol can normalize I_{Na} density in infarction-induced HF. We interpret these data not as a simple prevention of cell hypertrophy by the drug and normalization of cell size and NaCh density as a result, but rather carvedilol effects on NaCh expression. A recent study demonstrates that prolonged exposure to carvedilol can raise the number of cell surface voltage-gated NaChs in cultured bovine adrenal medullary cells [11] and supports our interpretation. This up-regulatory effect of carvedilol was thought to be associated with the drug effect on post-translational steps of NaChs synthesis, because authors did not find changes in mRNA encoding NaCh [11]. These findings led authors to hypothesize that carvedilol up-regulates NaChs by increasing vesicular trafficking from the trans-Golgi network, in which protein kinase A may be involved. On the other hand, abolishment of this mechanism may explain down-regulation of NaChs in chronic HF shown in the present study without the changes in mRNA [19].

The result of the recovery of I_{Na} density in chronic HF by long-term therapy with carvedilol (fig. 3) gives us a basis for another interesting speculation that improvement of heart performance produced by β-blockers might be related in part to a decrease in intra-ventricular delays [8, 23].

Speculations about mechanisms for the I_{Na} down-regulation and its recovery by carvedilol

Our previous studies showed that therapy with β-blockers in animals with experimentally induced HF significantly improves LV function and attenuates LV remodeling, recovers myocardial energetics, and arrests ongoing loss of cardiomyocytes through necrosis or apoptosis [for a review see ref. 24]. We previously reported that our canine HF model is characterized by a sustained increase in catecholamines [13] similar to that of HF patients. Thus, recovery of normal functional density of NaChs by carvedilol shown in the present study might be primarily related to protection of β-adrenoreceptors from overstimulation.

We speculate that both NaCh downregulation and the rescue effect of carvedilol found in our chronic HF model could be related to poor Ca^{2+} handling in the failing cells. Indeed, the primary action of β-adrenergic agonists is to increase the total Ca^{2+} influx through phosphorylated Ca^{2+} channels, which occurs on top of poor Ca^{2+} handling in HF [25]. The density of β-receptors was found to be decreased in different HF preparations including our HF model. [26] However, the residual β-adrenoreceptor responsiveness in HF myocardium is still critical for abnormal Ca^{2+} handling [e.g., spontaneous sarcoplasmic reticulum (SR) Ca^{2+} release] [27]. Up-regulated Na^+-Ca^{2+} exchange [27], increased activity of the L-type Ca^{2+} channel [28], and impaired function of SR [25] are distinctive features for chronic HF. The defective Ca^{2+} handling has also been found in our canine chronic HF model [29]. In turn, poor Ca^{2+} handling can trigger Ca^{2+}-related mechanisms for I_{Na} down-regulation (reported in rat cardiac muscle by Duff et al. [30]). Our experiments with BAPTA-AM (fig. 4) confirmed that intracellular Ca^{2+} could regulate I_{Na} in FVCs of

canine hearts. Other possible (although also speculative) mechanisms for an I_{Na} density decrease in our HF model include phosphorylation of NaChs by protein kinase C [31–33] and/or cytoskeleton alterations [17, 34, 35].

If the I_{Na} density decrease found in the present study is mediated by poor Ca^{2+} handling, chronic therapy with carvedilol can normalize the density by improving of Ca^{2+} handling in HF. Protection of β-adrenoreceptors from over-stimulation by carvedilol could decrease the total Ca^{2+} influx via L-type Ca^{2+} channels. Furthermore, β-blocker treatment has recently been shown to normalize the abundance of cardiomyocyte Ca^{2+} regulatory proteins, improve Ca^{2+} handling in HF patients [36], and restore cardiac Ca^{2+} release channel (ryanodine receptor) structure and function in a canine HF model [37]. We also found that chronic therapy with carvedilol improved cardiomyocyte contractility by improving SR function in our model dogs with HF [38].

Study limitations

It is important to note that we used carvedilol, a non-selective β-blocker with antioxidant properties [39]. Accordingly, we cannot distinguish contributions of different receptor subtypes and other mechanism(s) into the observed NaCh changes. Also, NaCh changes found in the present study were attributed to LV mid-myocardium from the apex region but not to the total LV. Thus the difference between our results and the previous results of unchanged I_{Na} density measured in total LV of the pacing canine HF model [40] can be explained by the HF origin (pacing vs multiple infarctions) as well as by local changes in NaCh expression.

In conclusion, experimental chronic HF results in down-regulation of the functional density of sodium channels in the left ventricle, which can be restored by chronic therapy with carvedilol, presumably via Ca^{2+}-dependent mechanism. The present findings may add new pharmacological actions of carvedilol to our understanding of the treatment of HF.

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