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Functional alterations after cardiac sodium-calcium exchanger overexpression in heart failure

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Münch, Götz, Kai Rosport, Christine Baumgartner, Zhongmin Li, Silvia Wagner, Andreas Bültmann, and Martin Ungerer. Functional alterations after cardiac sodium-calcium exchanger overexpression in heart failure. Am J Physiol Heart Circ Physiol 291: H488–H495, 2006. First published April 7, 2006; doi:10.1152/ajpheart.01324.2005.—The sodium-calcium exchanger (NCX) is discussed as one of the key proteins involved in heart failure. However, the causal role and the extent to which NCX contributes to contractile dysfunction during heart failure are poorly understood. NCX overexpression was induced by infection with an adenovirus coding for NCX, which coexpressed green fluorescence protein (GFP) (AdNCX) by ex vivo gene transfer to nonfailing and failing rabbit cardiomyocytes. Myocardial gene transfer in rabbits in vivo was achieved by adenoviral delivery via aortic cross-clamping. Peak cell shortening of cardiomyocytes was determined photo-optically. Hemodynamic parameters in vivo were determined by echocardiography (fractional shortening of cardiomyocytes was determined photo-optically). In nonfailing rabbits in vivo, basal systolic contractility (fractional shortening) and tip catheter [maximal first derivative of left ventricular (LV) pressure (dP/dt max)]; maximal negative derivative of LV pressure (−dP/dt max)]. Peak cell shortening was depressed after NCX gene delivery in isolated nonfailing and in failing cardiomyocytes. In nonfailing rabbits in vivo, basal systolic contractility (fractional shortening and dP/dt max) and maximum rate of LV relaxation (−dP/dt max) in vivo were largely unaffected after NCX overexpression. However, during heart failure, long-term NCX overexpression over 2 wk significantly improved fractional shortening and dP/dt max compared with AdGFP-infected rabbits, both without inotropic stimulation and after β-adrenergic stimulation with isoproterenol. −dP/dt max was also improved after adenovirus-mediated NCX overexpression in the failing rabbits group. These results indicate that short-term effects of NCX overexpression impair contractility of isolated failing and nonfailing rabbit cardiomyocytes. NCX overexpression over 2 wk in vivo does not affect myocardial contractility in nonfailing rabbits. Interestingly, in vivo overexpression of NCX decreased the progression of systolic and diastolic contractile dysfunction and improved β-adrenoceptor-mediated contractile reserve in heart failure in rabbits in vivo.

gene transfer

SODIUM-CALCIUM EXCHANGER (NCX) is one of the key regulators of calcium homeostasis and contractility in cardiomyocytes (3). Depending on the sodium and calcium gradient across the cell membrane and the membrane potential of the cell, NCX is able to transport calcium into the cell during systole (reverse mode) or to remove intracellular diastolic calcium levels during relaxation by outward transport (forward mode) (24). NCX is involved in the alterations of cardiac contractile function and calcium homeostasis in heart failure (7). Heart failure is characterized by decreased systolic calcium levels in the cardiomyocytes mainly due to depletion of intracellular calcium stores (4, 15) and slowed decrease of intracellular diastolic calcium levels due to depressed sarcoplasmic reticulum (SR) calcium uptake function. In patients with heart failure, NCX expression seems to be upregulated (7, 26). The role of NCX in heart failure, however, is not completely understood, and both beneficial effects (8) and deleterious effects of NCX in heart failure have been discussed (23). Animal models brought considerable insight into the role of NCX under chronic in vivo conditions (for review, see Ref. 20). Mice with conditional knockout of NCX (10) had a slight (20–30%) decrease of cardiac contractility and almost normal life spans. Transgenic animals with NCX overexpression (2.3-fold increased activity) presented with mild phenotypes (2, 27) and mainly myocardial hypertrophy. In contrast, overexpression of NCX in isolated cardiomyocytes from different species for hours or several days resulted mainly in deterioration of contractility of isolated cardiomyocytes (5, 21, 23).

It is unclear whether NCX upregulation is an epiphenomenon or whether it may cause functional alterations during heart failure. Thus we investigated the functional consequences of NCX overexpression in nonfailing rabbits and in a rabbit heart failure model, with dilative cardiomyopathy as found in human heart failure. The key findings of the rather acute, adenovirus-mediated NCX overexpression in isolated cardiomyocytes is that NCX overexpression depresses contractility of cardiomyocytes during increased stimulation frequencies. In contrast, long-term NCX protein overexpression in heart failure in vivo leads to an improvement of contractility and contractile reserve due to increased responsiveness to β-adrenergic stimulation and enhanced relaxation velocity. Long-term NCX overexpression in nonfailing rabbits in vivo had only minor effects on myocardial contractility and led to myocardial hypertrophy, in accordance with previous findings in transgenic mice.

MATERIALS AND METHODS

Construction and purification of recombinant adenovirus. cDNA of the canine NCX (18) was generated from a cardiac cDNA using PCR technique and specific primers containing the respective restriction sites. Specifically, a PCR product was generated with the 5′ primers GCGTT-TGAGTCCGATGCTGAGTTAAGACTATTCC containing a
BamHI restriction site and the specific dog NCX DNA sequence. The 3′ primer was designed GCCGGGTCTAGATTAGACCTTATAGTGCC for the specific dog NCX DNA sequence and the Xba I restriction site. The resulting construct was digested with the restriction enzymes Xba I and BamHI. The adenoviral shuttle vector pAdTrack-CMV (pAdTrack-CMV) was digested by Bgl II and Xba I. The cDNA of the NCX, including the suitable restriction sites, was then ligated into the pAdTrack-CMV plasmid. After homologues recombination by cotransfection of the pAdTrack-CMV containing the NCX cDNA with the adenoviral backbone plasmid pAdEasy, recombinants are selected. Recombinant (E1/E3 deficient) adenoviruses were generated by infection of HEK-293 cells with the recombinant plasmid, and large virus stocks were prepared, expressing NCX and green fluorescence protein (GFP) under control of two independent CMV promoters (AdNCX), according to the system of He et al. (9). As a control, adenovirus encoding for GFP only without further transgenes was used (AdGFP).

Preparation and culture of adult ventricular cardiomyocytes. Single calcium-tolerant ventricular myocytes were isolated from New Zealand White rabbits by collagenase type II digestion (175–200 U/ml; Cell Systems) as previously described (14). The infection of the ventricular cardiomyocytes with the adenoviruses was performed 6–8 h after plating in M199 culture medium [supplemented with Eagle’s minimum essential medium (MEM) vitamins 1:100; MEM nonessential amino acids 1:100; 10 μg/ml insulin, 100 IU/ml penicillin, and 100 μg/ml gentamicin]. Cardiomyocytes were kept in culture for a further 36 h before measurements were performed.

Immunoblots. Immunoblots from homogenates of cardiomyocytes isolated from failing rabbits and from myocardium of nonfailing and failing rabbits after gene transfer were performed according to Towbin et al. (27a) with modifications as previously described (5). Commercially available mouse monoclonal antibodies against canine NCX-1 protein (Abcam, Cambridge, MA), against bovine phospholamban (Upstate, Chicago, IL), against canine sarco(endo)plasmic reticulum Ca2+-ATPase 2a (SERCA2a), and against rabbit caldesmen-trin (ABR, Golden, CO) were used.

Immunohistochemistry of recombinant NCX. Histological slices of the heart and different organs from rabbits hearts harvested 2 wk after gene transfer were frozen. Detection of transgenic uptake-1 expression was done as previously described (17). Antibody binding was visualized with an avidin-biotinylated complex glucose oxidase (ABC-GO) system according to the manufacturer’s instructions (Vectastain ABC-GO Kit; Vector, Burlingame, CA).

NCX activity. Functionally relevant overexpression of NCX after gene transfer in cardiomyocytes was confirmed by measurement of sodium-dependent Ca2+ uptake into homogenates from cardiomyocytes according to the method of Reeves and Sutko (21a), with modifications as previously described (5). After washing procedures were performed, Ca2+ uptake was measured by radioactivity counting in a Beckman scintillation counter. NCX-specific uptake was calculated from the difference of 45Ca2+ uptake from NaCl-loaded homogenates minus the unspecific 45Ca2+ uptake from KCl-loaded homogenates.

Contraction experiments. The measurements of contraction amplitudes of AdGFP- or AdNCX-infected cardiomyocytes were carried out with the use of an electro-optical monitoring system as previously described (5). The experiments were performed with ventricular cardiomyocytes 36 h after adenoviral infection in a single-cell measuring device (Scientific Instruments, Heidelberg, Germany) in a temperature-controlled cuvette (37°C) at constant medium flow of 0.5 ml/min using 1.8 mmol/l Ca2+-Tyrode solution.

Model of heart failure. Pacemakers from Vitatron (Düsseldorf, Germany) were implanted into New Zealand White rabbits (weighing 3.6 ± 0.3 kg; Asam, Kissing, Germany). Rapid pacing was initiated 6 h after gene transfer at 320 beats/min for 1 wk and further increased to 360 beats/min for one more week as previously described (17).

Adenoviral gene transfer to rabbit myocardium. Both nonfailing and failing rabbits due to rapid left ventricular (LV) pacing received adenoviral gene transfer (4 × 1010 plaque-forming units) to the myocardium by aortic and pulmonary cross-clamping as previously described (17). Adenoviral gene transfer was performed at day 0 directly after the initial echocardiography measurement of cardiac function. In the two groups, eight rabbits each received AdGFP for control or AdNCX as the gene of interest. For all interventions, the rabbits were anesthetized with fentanyl (0.01 mg/kg) and propofol (2%; 80 mg·kg−1·h−1). All animal experiments were conducted according to the animal protection and safety regulations of the State of Bavaria. The protocol has been reviewed, and allowance was given by the State of Bavaria in the application for animal experiments no. 21–2531-43/01.

Measurement of cardiac function and LV hemodynamics. LV contractility and dimensions were measured by echocardiography at day 0 just before and 7 and 14 days after adenoviral gene delivery both in nonfailing and failing rabbits as previously described (17).

At day 14 after adenoviral gene transfer, both in nonfailing rabbits and in the rabbits with rapid ventricular pacing, a final LV catheterization was carried out with a 3-Fr micrometer-tipped catheter, as described in detail elsewhere (17).

Measurement of heart rate, R-R interval, and QT time. Heart rate and ECG monitoring was carried out continuously by an implanted ECG sensor and continuous transmission by telemetry (PhysioTel Implant, DSL, Transoma Medical, St. Paul, MN). With the use of DSI technology (Dataquest ART, DSI) and EMKA ECG-software (ECG-Auto, EMKA Technologies, Paris, France), the acquisition and analysis of heart rate and R-R interval were carried out in the rabbits at baseline and after the gene transfer, continuously for 2 wk. QT time was measured in the animals without rapid pacing during 1 h at baseline and for 1 h at 1- and 2 wk after the gene transfer.

Data analysis. All experiments were performed in eight animals or with at least 30 isolated cardiomyocytes. The analysis of data was carried out in a blinded manner. All data are expressed as means ± SE. For statistical analysis, one-way ANOVA for independent measurements was used. For all analyses, a value of P < 0.05 was considered to be statistically significant.

RESULTS

NCX protein overexpression in isolated cardiomyocytes after adenoviral infection. NCX overexpression in cardiomyocytes ex vivo was achieved in isolated cardiomyocytes from nonfailing and failing rabbit hearts by adenoviral infection with AdNCX. Infection of isolated rabbit cardiomyocytes with AdNCX with a multiplicity of infection (MOI) of 10 leads to a significant overexpression of the NCX protein compared with AdGFP infected failing controls (Fig. 1, A and B). The transgenic NCX protein was functionally active and led to an increase in the NCX-dependent calcium uptake activity in the AdNCX-infected cardiomyocytes (MOI of 10) (see Fig. 1C). NCX-dependent calcium uptake was blunted by the addition of the NCX blocker KB-R 7943 (10 μmol/l).

Single-cell contractility of cardiomyocytes after NCX gene transfer. The infection of nonfailing cardiomyocytes with AdNCX (MOI of 10) led to a decreased frequency-dependent systolic contractility (peak cell shortening; Fig. 2, left). The infection of failing cardiomyocytes with AdNCX (MOI of 10) also resulted in a depression of the frequency-dependent systolic contractility (Fig. 2, right).

Heart failure in vivo. Rapid pacing was used to induce heart failure in rabbits. Myocardial contractility as quantified by echocardiography [LV fractional shortening (FS), in %] declined from 36.8 ± 1.2 before the onset of pacing to 27.7 ± 0.7 after 7 days and to 22.0 ± 0.5% after 14 days. After 14 days, the systolic contractility +dP/dtmax, as determined by invasive...
gene transfer (Fig. 3A). For quantification of other crucial calcium-cycling proteins, immunoblot were performed from preparations of the identical hearts and compared with preparations from nonfailing rabbit hearts. The means ± SE of the densitometric evaluation of immunoblots from myocardial preparations after normalization to calsequetrin are shown in Fig. 3B. The evaluation of myocardial slices for GFP expression showed homogenous GFP coexpression throughout the left ventricle, whereas no signal occurred in noninfected regions of the septum and right ventricle of the heart (Fig. 3C). Transgenic NCX protein can be localized in the myocardium of rabbits after adenoviral gene transfer (Fig. 3D). Transgene expression was significantly present in the spleen but not in other organs, such as liver or kidney, or in calf muscle after in vivo adenoviral gene delivery.

Effects of NCX gene transfer on LV function in nonfailing rabbits and rabbits with rapid LV pacing developing heart failure. LV function, as determined by echocardiography (FS), was not significantly decreased in the NCX-expressing nonfailing rabbits compared with the AdGFP-infected nonfailing rabbits (after 1 wk: AdGFP 36.3 ± 1.6 vs. AdNCX 34.1 ± 1.7%; at 2 wk after gene transfer: AdGFP 33.4 ± 1.5 vs. AdNCX 34.0 ± 0.9%). In contrast, the mean wall thickness was significantly increased in the AdNCX infected nonfailing rabbits (AdGFP 0.27 ± 0.02 vs. AdNCX 0.30 ± 0.01 cm; P < 0.001). In paced rabbits with NCX overexpression, FS was significantly improved during the progression of heart failure compared with AdGFP-infected rabbits, both 1 and 2 wk after gene transfer (Fig. 4). In contrast, the mean wall thickness did not differ between the two groups (AdGFP, 0.25 ± 0.01 vs. AdNCX, 0.24 ± 0.01 cm).

At day 14 after adenoviral gene delivery by aortic cross-clamping in the NCX-expressing nonfailing rabbits, the basal dP/dt max was not different from the AdGFP-infected rabbits (AdGFP 2,855 ± 472 vs. AdNCX 2,165 ± 368 mmHg/s). The dP/dt max after isoproterenol stimulation was also not significantly different in both groups at variable doses of isoproterenol (e.g., at 0.5 μg/kg, AdGFP 7,670 ± 1,225 vs. AdNCX 5,058 ± 946 mmHg/s). The LV end-diastolic filling pressure 14 days after gene transfer was also similar in both groups of healthy rabbits (AdGFP, 3.82 ± 0.6; AdNCX, 1.94 ± 0.7 mmHg).

In the rabbits with heart failure after NCX-expression, basal dP/dt max was significantly higher than in the AdGFP-infected control group. In the NCX-overexpressing group with heart failure, the dP/dt max after isoproterenol stimulation was also significantly improved at 0.5 and 1.0 μg/kg of isoproterenol, indicating an improvement in contractile reserve (Fig. 5A). The maximal relaxation (−dP/dt max) was also significantly improved in the NCX-overexpressing group of failing rabbit hearts (Fig. 5B). Under isoproterenol, there was only a nonsignificant difference of −dP/dt max after NCX overexpression (Fig. 5B). The LV end-diastolic filling pressure at the end of the heart failure protocol was similar after NCX and GFP gene transfer in the failing rabbits (Fig. 5C).

Influence on heart rate and QT duration after NCX overexpression. Heart rate was not significantly increased in AdNCX nonfailing rabbits compared with AdGFP-infected nonfailing rabbits [basal: AdGFP, 186 ± 9 vs. AdNCX, 208 ± 5 beats/min; isoproterenol (1.0 μg/kg): AdGFP, 312 ± 11 vs. AdNCX, 330 ± 9 beats/min]. In failing rabbits, the basal heart rate (AdGFP, 197 ± 3.2 vs. AdNCX, 198 ± 2.6 beats/min) and

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**Fig. 1.** Effect of sodium-calcium exchanger (NCX) overexpression after adenoviral delivery of green fluorescence protein (GFP; AdGFP) or NCX (coexpressed with GFP; AdNCX) to isolated cardiomyocytes in vitro. A representative Western blot shows increase of NCX expression after adenoviral delivery to isolated cardiomyocytes. Control infection with AdGFP and uninfected cardiomyocytes is shown for comparison. B: quantification of NCX protein expression by densitometry is presented. Means ± SE of NCX expression in cardiomyocytes normalized to calsequetrin after infection with 10 multiplicity of infection of AdNCX are demonstrated. AdGFP-infected and -uninfected failing cardiomyocytes serve as controls. C: NCX-dependent calcium uptake was determined by sodium-driven calcium uptake measurement in cardiomyocytes. Time-dependence of NCX-specific calcium uptake is shown in cardiomyocytes infected with 10 plaque-forming units (pfu)/cell AdNCX compared with GFP-expressing failing control cardiomyocytes (AdGFP). Data represent means ± SE of cardiomyocytes from 8 different animals with triplicate measurements. *Significance of P < 0.05 AdNCX vs. AdGFP.

Pressure-tipped catheter, was 1,550 ± 130 mmHg/s in failing hearts (vs. 3,100 ± 250 mmHg/s in healthy controls; P < 0.05). LV end-diastolic pressure increased from 3.7 ± 0.3 at day 0 to 6.4 ± 0.9 mmHg after 14 days (P < 0.05).

NCX overexpression after adenoviral delivery in the myocardium in vivo. Immunoblotting documented increased expression of NCX at the end of the observation period 2 wk after...
heart rate after stimulation with isoproterenol (1.0 μg/kg: AdGFP, 322 ± 5.4 vs. AdNCX, 323 ± 5.6 beats/min) were similar in both groups. In the NCX group, no signs of increased mortality and no increase in sudden cardiac death were observed compared with the GFP control rabbits. The QT interval was analyzed as a parameter of action potential duration by ECG and telemetry. In the nonfailing rabbits, no difference between AdNCX and AdGFP infection was observed. In rabbits with rapid pacing, after 7 and 14 days, the relative QT time was significantly longer in the NCX-transduced group compared with the GFP control group (7 days: AdGFP, 61.4 ± 1.3 vs. AdNCX, 64.4 ± 1.1% of R-R interval; P < 0.01; 14 days: AdGFP, 59.6 ± 0.8 vs. AdNCX, 64.1 ± 2.2% of R-R interval; P < 0.01).

DISCUSSION

The crucial findings of this in vivo and in vitro assessment of NCX overexpression in nonfailing rabbits and pacing-induced rabbit heart failure are the following. Short-term NCX overexpression in isolated failing and nonfailing cardiomyocytes leads to a blunted frequency-dependent systolic contractility. In contrast, LV systolic contractility, contractile reserve, and LV relaxation in vivo are significantly improved during the progression of heart failure by long-term NCX overexpression. However, NCX overexpression over 2 wk in nonfailing rabbits had a prohypertrophic effect on the myocardium without significant effects on myocardial contractility.

Functional effects of NCX overexpression in isolated cardiomyocytes. Several groups have found a depressed frequency-dependent inotropic response after NCX overexpression in isolated nonfailing cardiomyocytes (5, 23). These findings were confirmed in the present study. The mechanistic background of these changes in cardiomyocyte contractility probably related to intracellular calcium depletion as previously described (23). In failing cardiomyocytes, increased expression of NCX also led to an impairment of the systolic contractility on increased stimulation frequency. Hobai and colleagues (11) recently showed that partial inhibition of NCX by the inhibitory protein XIP had a beneficial effect in isolated cardiomyocytes from a canine heart failure model. The results of the present study with overexpression of NCX at a MOI of 10 AdNCX, which led to an impairment of systolic contractility, and the observations of Hobai and colleagues would thus favor a short-term inhibition of NCX for an improvement of systolic contractility of failing cardiomyocytes. However, the respective levels of NCX overexpression or the levels of NCX inhibition might critically determine the consequences of NCX modulation on myocardial function.

Functional effects of NCX overexpression in myocardium in vivo. In the present study, NCX overexpression in nonfailing rabbits in vivo led to myocardial hypertrophy without significant effects on myocardial contractility. Previous studies in transgenic mice with mild overexpression of the NCX described unchanged or increased calcium flux in the systole and diastole via increased forward- and reverse-mode NCX activity (27, 30), resulting in a minor improvement of contractile function. In both models, NCX overexpression induced myocardial hypertrophy. Species differences account for the different contributions of NCX to the overall calcium transient and the calcium transport direction of NCX, depending on the intracellular sodium concentration and on action potential characteristics. However, the overall contribution of NCX to the cardiac function in nonfailing myocardium seems to be of minor significance in both models.

Interestingly, cardiac NCX overexpression over the period of 2 wk in rabbits in vivo decreased the rate of progression of heart failure. Improvement of contractile function after NCX overexpression during heart failure does not seem to be a consequence of alterations of other calcium-cycling proteins. For example, SERCA2 expression and phospholamban showed no statistically significant changes after NCX overexpression in failing rabbit hearts. Thus NCX might improve calcium homeostasis in the systole by increased reverse-mode activity. Very recently, NCX overexpression was investigated in transgenic mice under pathophysiological conditions of cardiac hypertrophy (25). These authors described that NCX overexpression in transgenic animals compensated for most of the key features of hypertrophy: the prolongation of calcium transients and action potential duration was reversed, and the SR calcium content was enhanced by NCX overexpression. The beneficial effect of NCX overexpression on contractility in cardiac hypertrophy and failure could be explained by increased forward- and reverse-mode NCX activity in the myocardium of the rabbits during heart failure after adeno viral gene transfer. Accordingly, the alterations in calcium homeostasis during heart failure, which is characterized by reduced systolic intracellular calcium and prolonged calcium transients in the diastole, could be partially compensated by long-term NCX overexpression leading to removal of the intracellular calcium during diastole by forward-mode NCX activity. Enhanced reverse-mode NCX activity could also improve calcium homeostasis in the myocardium during systole after NCX protein overexpression. This beneficial effect of increased NCX activity, however, might depend on the alterations in the myocardium found during heart failure, such as impaired SR function. Moreover, alterations in the phosphorylation status of NCX in
Fig. 3. Effect of adenoviral gene transfer of either GFP (AdGFP) or NCX (coexpressed with GFP; AdNCX) in rabbits in vivo. A: NCX expression in rabbit myocardium after gene transfer with $4 \times 10^{10}$ pfu AdNCX or $4 \times 10^{9}$ pfu AdGFP per rabbit using aortic cross-clamping was determined by immunoblotting from myocardial homogenates. A representative experiment is shown comparing NCX expression in myocardium after AdNCX adenoviral delivery in vivo with AdGFP control delivery (A, left). Means ± SE of densitometry of NCX expression are shown in summary (right). B: quantification of protein expression of sarco(endo)plasmic reticulum Ca$^{2+}$-ATPase (SERCA2) and phospholamban was performed by immunoblotting of myocardial preparations of nonfailing rabbits and of rabbits with heart failure 14 days after infection with AdNCX or AdGFP by aortic cross-clamping in vivo. Protein expression of SERCA2 and phospholamban was normalized to calsequestrin. Nonfailing rabbit hearts served as controls. All measurements were done in myocardium from 8 animals with triplicate measurements. *Significance of $P < 0.05$ AdNCX vs. AdGFP. C: expression of transgene 14 days after adenoviral aortic cross-clamping in vivo is verified by GFP-specific fluorescence in myocardial slices viewed under ultraviolet light illumination. Representative macroscopic views of myocardial cross sections of AdGFP (left) and AdNCX (coexpressing GFP; right) infected animals are shown. D: localization of transgenic NCX expression in myocardium is demonstrated by immunohistochemistry of myocardial histological sections after in vivo transfection with AdNCX and AdGFP in rabbits with heart failure.
heart failure could also contribute to the degree of functional alterations in these failing hearts. As published recently, calcineurin, a phosphatase and important modulator of protein function during heart failure (16), also inhibits NCX function (13). Thus the degree of compensation of myocardial function by NCX overexpression could partially be evened out by NCX dephosphorylation during the progression of heart failure in the pacing rabbit model. In nonfailing myocardium in rabbits in vivo, with a balanced calcium homeostasis, NCX overexpression had no significant effect on contractile function.

**Differences between acute and chronic effects of NCX overexpression.** Long-term NCX overexpression in vivo and acute NCX overexpression in isolated cardiomyocytes lead to different effects on contractility. This is a phenomenon that is well known in heart failure, e.g., for the effects of a therapeutic β-adrenoceptor inhibition in patients with heart failure. Initial deterioration of myocardial function after β-adrenergic inhibition is followed by a pronounced improvement of the long-term outcome of patients with heart failure (6). Thus adenoviral overexpression in isolated cardiomyocytes seems to allow for the functional study of short-term effects of NCX overexpression. Long-term effects of NCX overexpression, however, can be studied by adenoviral gene transfer in rabbits in vivo. In the present study, gene transfer to failing cardiomyocytes infected in vitro could serve as a potential “rescue” model for gene transfer to failing cardiomyocytes, whereas the present model of in vivo gene transfer before the rapid LV pacing seems a suitable model for the prevention of heart failure by gene transfer or for the influence of long-term gene transfer on the progression of heart failure in an animal model of heart failure.

**Clinical implications.** The prevention of cardiac dysfunction during heart failure in the present model is in accordance with previous observations in patients with heart failure (28, 29). However, the authors of these studies concluded that the increased reverse-mode NCX activity aggravates diastolic dysfunction by the increase of systolic calcium concentrations on a background of impaired SR function. In contrast, the present findings suggest that NCX overexpression also compensates diastolic function by increased forward mode. In an intriguing study (8) in explanted hearts from patients with heart failure, the hypothesis that increased NCX expression might possibly compensate for depressed SERCA activity in heart failure was
outlined. These investigators showed a positive correlation between the extent of NCX protein expression and the improvement of diastolic function and frequency-dependent increase of contractility (8). The conclusion from these findings was that NCX might contribute to the removal of intracellular calcium in cardiomyocytes via an increased forward-mode activity in the diastole. Thus, with increased NCX expression, either by gene transfer or by the increase of protein expression as observed in heart failure patients (8), the impaired calcium cycling could be compensated by NCX, leading to improvement of the contractile reserve by improving diastolic function, the force-frequency relationship, and the positive inotropic response to sympathetic nerve activation. The situation in the nonfailing heart seems to be rather different, however, because NCX overexpression in nonfailing rabbits in vivo resulted in hypertrophy with no significant effect on myocardial contractility. On the basis of a well-balanced calcium homeostasis, changes in calcium transport via NCX across the cell membrane, either outward or inward calcium transport, do not seem to have an impact on the myocardium under physiological conditions.

In summary, our in vivo findings corroborate previous findings in the myocardium from patients with heart failure (8, 28). These findings show the relevance of NCX for the regulation of cardiac contractility in heart failure. A stimulator of NCX would compensate for decreased functions of other calcium regulatory proteins, e.g., SERCA2a, and could be beneficial in heart failure. However, the amount of NCX stimulation, the native expression levels of NCX, and also the function of the SR might also play important roles in determining the functional consequences of NCX modulation. Moreover, the development of an efficient and safe drug for the treatment of heart failure might be further complicated by the arrhythmogenic potential of a stimulation of the NCX (1). Thus further studies are needed to understand the cellular changes after NCX overexpression in calcium homeostasis, action potential duration, and electrical activity, among others in failing myocardium.

Limitations of the study. Various animal models have shown great similarity in the hemodynamic and pathophysiological characteristics compared with the situation in human heart failure, including upregulation of NCX during heart failure (12, 19). Even if most characteristics are highly comparable between the pacing-induced rabbit heart failure model and human heart failure, upregulation of NCX could not be shown in one investigation of the rabbit model (31), whereas others report NCX upregulation in a rabbit heart failure model (22). Moreover, species differences additionally complicate the interpretation for the relevance in humans. However, the measurement of hemodynamics in intact animals in vivo comparing overexpression of NCX to the respective controls should allow to improve the understanding of NCX function in heart failure.

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