

Effects of α -adrenergic stimulation on the sarcolemmal $\text{Na}^+/\text{Ca}^{2+}$ -exchanger in adult rat ventricular cardiocytes

Hans Reinecke^{a,*}, Roland Vetter^b, Helmut Drexler^a

^a *Universität Freiburg, Innere Medizin III, Kardiologie und Angiologie, Berlin-Buch, Germany*

^b *Max-Delbrück-Centrum für Molekulare Medizin (MDC), Berlin-Buch, Germany*

Received 17 February 1997; accepted 6 June 1997

Abstract

Objective: The cardiac sarcolemmal $\text{Na}^+/\text{Ca}^{2+}$ -exchanger (NCX) plays an important role in the maintenance of the myocardial Ca^{2+} homeostasis which is altered in cardiac hypertrophy and failure. The aim of the present study was to investigate whether α -adrenergic stimulation known to induce cardiac hypertrophy might be involved in the regulation of the sarcolemmal NCX. **Methods:** Adult rat ventricular cardiocytes (ARC) were isolated from male Sprague–Dawley rats. Phenylephrine, an α -adrenergic agonist, was used as hypertrophic agent. NCX expression was measured by competitive RT-PCR and Western blot analysis. **Results:** α -Adrenergic stimulation of ARC with 10 μM phenylephrine for 24 h resulted in a significant increase of the NCX mRNA (2.5-fold) and the NCX protein level (1.8-fold). The changes on the expression level were blocked by the α_1 -adrenoceptor antagonist prazosin. **Conclusions:** The data demonstrate that the NCX expression level is up-regulated by the activation of the α -adrenergic signal transduction pathway. The increased NCX mRNA level induced by α -adrenergic stimulation appeared to be translated into an increased NCX protein level. © 1997 Elsevier Science B.V.

Keywords: $\text{Na}^+/\text{Ca}^{2+}$ -exchanger; Rat; Cardiocytes; α -adrenoceptor; Expression

1. Introduction

The pathophysiology of heart failure is closely associated with neuroendocrine changes [1]. These changes involve the activation of the adrenergic system leading to increased plasma concentrations of catecholamines and, thereby, serving as a compensatory mechanism for the failing circulation. However, overshooting activation is likely to have negative long-term effects [2]. Therefore, it is worth to investigate whether disturbances of the myocardial Ca^{2+} homeostasis, which occur in experimental cardiac hypertrophy as well as in human heart failure [3], are affected by catecholamines.

The cardiac sarcolemmal $\text{Na}^+/\text{Ca}^{2+}$ -exchanger (NCX) plays an important role in the control of Ca^{2+} fluxes across the sarcolemma of the cardiomyocyte and, there-

fore, in the control and maintenance of the myocardial Ca^{2+} homeostasis [4,5]. Previous studies have presented evidence for an increase of the NCX gene expression and/or activity in animal models of pressure-overload induced hypertrophy [6,7]. Studies from our laboratory have shown that the NCX gene expression and the functional activity of the antiporter are increased in patients with severe heart failure [8,9]. It was hypothesised that the increased NCX expression and function in failing hearts might, in part, compensate for depressed sarcoplasmic reticulum (SR) Ca^{2+} -ATPase function, implying that an increased NCX expression in the failing heart might limit intracellular Ca^{2+} overload during diastole and improve relaxation [8,9].

Several experimental studies have shown that cardiac hypertrophy could be initiated and maintained by chronic infusion of adrenergic agonists (for a review, see [10]). In vitro studies using neonatal cardiocytes have clearly

* Corresponding author. University of Washington, Department of Pathology, Box 357470, Room E520 HSB, Seattle, WA 98195-7335, Tel. (+1-206) 6168684; Fax (+1-206) 5433644; E-mail: hreineck@u.washington.edu

demonstrated load-independent hypertrophic effects of α_1 -adrenergic agonists. The growth effect was characterised by increased rates of protein synthesis, myocyte surface area, protein content, total transcriptional activity [10], and induction of protooncogene gene expression [11,12]. The hypertrophic response to α_1 -adrenergic stimuli also involved the selective up-regulation of the early developmental contractile protein isogenes, skeletal α -actin, and β -myosin heavy chain genes [13–15]. Increased protein synthesis due to α_1 -adrenoceptor stimulation was also shown for adult rat cardiocytes [16]. However, regarding a transcriptional activation by α_1 -adrenergic agonists the data are inconsistent [16–18]. α_1 -Adrenergic activation is known to activate protein kinase C [19], and to generate increased levels of inositol trisphosphates, which in turn induce the release of Ca^{2+} from intracellular stores [20,21]. Since those effects were blocked by the α_1 -specific antagonist prazosin they are likely to be mediated by α_1 -adrenoceptor subtype. In addition, there is no evidence for an α_2 -adrenoceptor subtype in rat cardiac myocytes [22,23].

In summary, α_1 -adrenergic agonists were shown to exert potent hypertrophic effects on cardiocytes. The goal of the present study was to investigate whether α_1 -adrenergic stimulation might be involved in the regulation of the sarcolemmal NCX expression in isolated adult rat cardiocytes.

2. Methods

2.1. Preparation of adult rat ventricular cardiocytes (ARC)

Ca^{2+} -tolerant ARC were isolated according to Powell [24] with modifications of Kammermeier and Rose [25]. Briefly, hearts were rapidly removed from adult male Sprague–Dawley rats (SAVO, Kisslegg, FRG) weighing 380 to 440 g following pentobarbital anaesthesia (80 mg/kg), washed in Ca^{2+} -free perfusion buffer (PB) (128.2 mM NaCl, 4 mM KCl, 0.19 mM NaH_2PO_4 , 1.01 mM Na_2HPO_4 , 1.39 mM MgSO_4 , 10 mM HEPES, 5.5 mM D-glucose, 2 mM pyruvate, 12.5 $\mu\text{g}/\text{ml}$ gentamycin, pH 7.4). Hearts were retrogradely perfused with PB, followed by perfusion with enzyme solution containing 40 U collagenase A and 0.1% hyaluronidase (Boehringer Mannheim). After removing the atria and great vessels, the ventricular tissue was finely minced and incubated in PB plus 2% albumin. CaCl_2 was added stepwise to the cell suspension to achieve a final concentration of 1 mM CaCl_2 . The cell suspension was filtered through a 300 μm mesh, and ARC were collected by centrifugation ($25 \times g$, 2 min). To reduce nonmyocyte contamination, the resulting pellet was resuspended (PB, 2% albumin, 1 mM CaCl_2), carefully overlaid onto a 6% albumin solution (in PB, 1 mM CaCl_2) and centrifuged. The cell pellet was resuspended in serum-free M-199 (Sigma) supplemented with L-carnitine (2 mM), creatine (5 mM), taurine (5 mM), albumin (0.2%),

penicillin (100 U/ml) and streptomycin (100 $\mu\text{g}/\text{ml}$). The cells were plated onto laminin-coated (1 $\mu\text{g}/\text{cm}^2$) 60 mm cell culture dishes (Falcon) at a density of $2 \times 10^5/\text{dish}$ and incubated for 2 h (5% CO_2 -incubator). The use of laboratory animals for scientific purposes was approved by the ethical committee of the University of Freiburg and the investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

2.2. Stimulation of ARC

For α -adrenergic stimulation the medium was replaced by medium supplemented with PE (10 μM), PE (10 μM) + PRAZ (20 μM) or PRAZ (20 μM). The influence of general trophic effects was tested in control experiments ($n = 5$) by the addition insulin (0.1 μM) to the culture medium. In blocking experiments, the antagonist was given 30 min prior to the agonist. The density of ARC after the medium change was 1 to 1.5×10^3 ARC/ cm^2 with about 90% rod-shaped cells. PE and PRAZ were purchased from Sigma, dissolved and stored according to the supplier's instructions. 24 h experiments were performed without medium change. When ARC were incubated for 72 h, the medium was changed every 24 h.

2.3. RNA preparation

ARC were washed twice with phosphate-buffered saline (PBS) and total cellular RNA was isolated according to the method of Chomczynski and Sacchi [26] and quantitated in triplicate by absorbance at 260 nm. The integrity of the RNA was checked by agarose gel electrophoresis prior to PCR analysis.

2.4. Competitive RT-PCR

The determination of the original number of NCX transcripts was performed by competitive RT-PCR in the presence of a defined concentration of a shortened NCX competitor RNA (ΔNCX) which served as an internal standard. The protocol was previously described in detail for the quantitation of the angiotensin I-converting enzyme [27]. Equal amounts of total RNA (150 ng) were mixed with increasing quantities of ΔNCX molecules (50 to 1.25×10^5) in 1x RT buffer (50 mM Tris-HCl pH 8.3, 75 mM KCl, 3 mM MgCl_2) completed with 0.5 mM dNTPs, 250 pmol of random hexanucleotide primers, 10 mM dithiothreitol, RNase inhibitor (20 U/100 ng total RNA; Amersham Buchler, Braunschweig, FRG) and MMLV reverse transcriptase (10 units/100 ng total RNA; Life Technologies, Eggenstein, FRG). The RT reactions (25 μl) were performed by incubation at 42°C for 60 minutes.

Duplicate samples of PCR reaction were performed as described [27]. Denaturing, annealing, and extension reactions proceeded 30 times at 94°C for 1 min, 60°C for 2

min, and 72°C for 3 min. As a negative control, no amplification product occurred if reverse transcriptase or total RNA was omitted in the first-strand cDNA reaction. The NCX PCR products were found to be of the expected size as shown by gel electrophoresis. The amplification products were separated by agarose gel electrophoresis (1.5%), stained with ethidium bromide and photographed under UV transillumination. The negative film was used to evaluate the band densities (Personal Densitometer, Molecular Dynamics, Krefeld, FRG). To correct for differences in size of the target (861 bp) and competitor (778 bp) PCR products, the band densities of the respective competitor PCR products were multiplied by the specific factor 1.1.

2.5. Selection and synthesis of the PCR primers

Sense and antisense primer oligonucleotides were selected from the rat NCX cDNA sequence [28] which is identical to the guinea pig [29] cDNA sequence in the region of the selected primers (sense: 1981–2001; 5'-AATGAGCTTGGTGGCTTACA-3' and antisense: 2827–2844; 5'-CCGCCGATACAGCAGCAC-3').

2.6. Construction and *in vitro* transcription of the competitor templates

For construction of internal standard competitor RNA a fragment of 83 bp was released by digestion of the NCX cDNA with *HpaI* and *BclI*. The shortened Δ NCX cDNA fragment was filled with the Klenov fragment and religated. For *in vitro* transcription, the shortened Δ NCX cDNA clone was linearised with the restriction enzyme *XhoI*. Then, 1 μ g of the digested Δ NCX cDNA template was transcribed into RNA by using a T3/T7-RNA polymerase *in vitro* transcription kit (Stratagene, Heidelberg, FRG). Subsequently, the DNA template was removed by DNase digestion. The competitor RNA template was purified by phenol extraction and quantitated by absorption at 260 nm.

2.7. Northern blot analysis

Northern blot analysis for the NCX was performed as described previously [8,33]. For the detection of the NCX mRNA, 20 μ g total RNA were separated by agarose gel electrophoresis, transferred to a nitrocellulose membrane and hybridised with a specific 1.5 kb guinea pig NCX cDNA fragment (generous gift from Dr. K.D. Philipson) and a 1.2 kb chicken glyceraldehyde dehydrogenase (GAPDH) fragment. The blot was washed as described [33] and exposed to X-ray film (Kodak Inc.) using intensifying screens (Siemens).

2.8. Western blot analysis

After incubation with hormones, cells were washed with PBS, scraped off into HEPES buffer (20 mM HEPES,

4 mM EGTA, 1 mM dithiothreitol; pH 7.4) completed with protease inhibitors (0.1 mM leupeptin, 0.3 mM PMSF). Preparation of particular protein fractions (i.e. crude membrane protein), SDS–polyacrylamide gel electrophoresis, and the immunodetection of the NCX protein was performed as previously described in detail [8,9]. 60 μ g of particular fraction protein were loaded (7.5% running gel) and subsequently transferred to Hybond-nitrocellulose (Amersham). Equal loading was checked by reversible staining of the membrane with Ponceau S. The immunoreaction was performed as described [8,9]. The polyclonal rabbit- α -dog NCX antiserum was purchased from Swiss Antibodies Swant Ltd. (Bellinzona, Switzerland). Quantification of the immunoreactive bands was performed by using a Personal Densitometer and the ImageQuant software (Molecular Dynamics, Krefeld, Germany).

2.9. Statistical analysis

All data are given in mean \pm SEM. Differences between groups were evaluated by analysis of variance (ANOVA) followed by Student-Newman-Keuls test. Statistical significance was accepted at the level of $p < 0.05$.

3. Results

Northern blot analysis was used to evaluate whether α_1 -adrenergic stimulation induced changes on the NCX expression level. Fig. 1 shows that PE-stimulation for 24 h induced a marked increase of the NCX hybridisation signal. The effect of PE appeared to be blocked by preincubation of ARC with the α_1 -specific adrenoceptor antagonist PRAZ (Fig. 1). The blocking effect of PRAZ indicates a specific α_1 -adrenoceptor mediated response to PE. Prazosin alone did not increase the expression of the NCX (Fig. 1).

3.1. Competitive RT-PCR analysis

The original number of NCX transcripts was determined by competitive RT-PCR analysis using the same

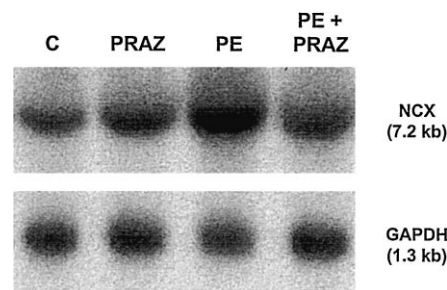


Fig. 1. Northern blot analysis of the NCX mRNA in ARC treated for 24 h with phenylephrine (PE), phenylephrine + prazosin (PE + PRAZ), and prazosin alone (PRAZ). Control = C.

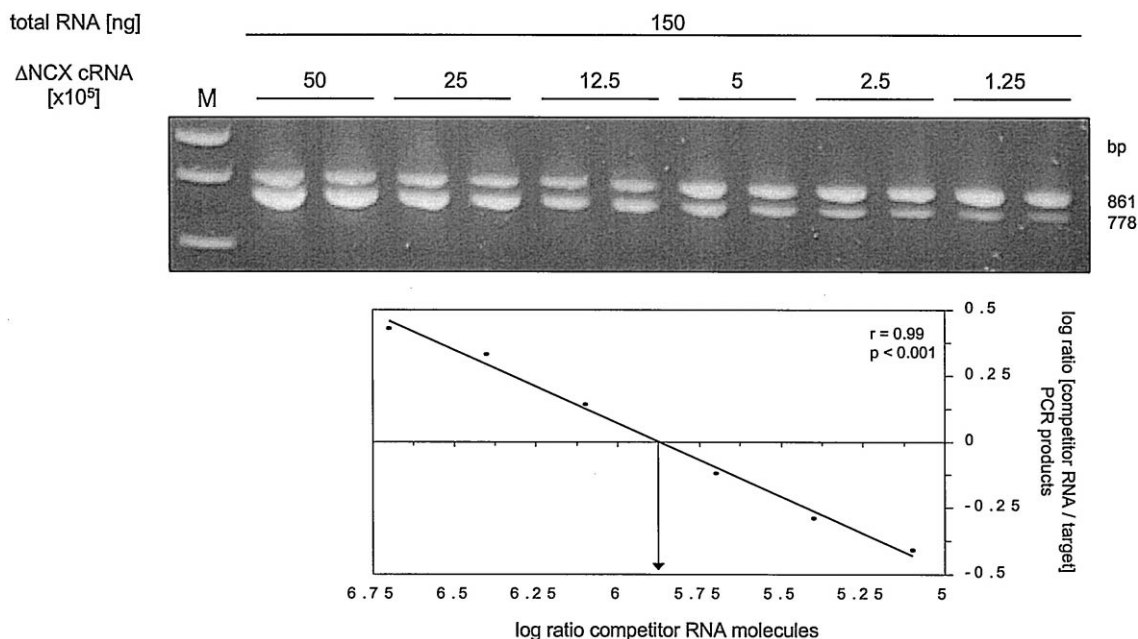


Fig. 2. NCX mRNA quantification by competitive RT-PCR. (Top) A constant amount of total RNA was mixed with an increasing number of Δ NCX standard molecules, reverse transcribed and amplified by PCR. The PCR products (NCX target: 861 bp, Δ NCX standard: 778 bp) were separated, stained and photographed under UV transillumination. The negative film was used to evaluate the band densities. (Bottom) The ratio of competitor-to-target products was plotted against the known number of competitor molecules on a log scale. At the competition equivalence point (log ratio = 0) the original number of target mRNAs corresponds to the initial number of competitor RNA molecules used. M = λ /HaeIII DNA molecular weight marker.

source of mRNA as above and a shortened fragment (Δ NCX) of the original NCX cDNA as an internal standard (Fig. 2). PE-stimulation induced a significant increase of NCX transcripts after 24 h ($n = 11$; $18.84 \pm 3.29 \times 10^5$ /60 ng total RNA) and 72 h ($n = 5$; $16.66 \pm 3.42 \times 10^5$ /60 ng total RNA), respectively, as compared to controls (24 h: $7.59 \pm 1.92 \times 10^5$ and 72 h: $5.31 \pm 1.07 \times 10^5$ /60 ng total RNA; both $p < 0.05$ vs. control) (Fig. 3). The effect of PE was completely blocked by preincubation of ARC with PRAZ and subsequent incubation with PE (24 h: $8.41 \pm 2.62 \times 10^5$ and 72 h: $4.08 \pm 0.92 \times 10^5$ /60 ng total RNA; both $p < 0.05$ vs. PE and NS vs. control) (Fig. 3). No changes of the NCX mRNA level were found, when ARC were incubated with PRAZ alone (data not shown).

To control for general trophic effects (overall increase of protein turn-over), the effect of insulin was tested ($n = 5$) (Fig. 3 and Fig. 4). Importantly, insulin, which was shown to increase the rates of general protein synthesis in adult rat cardiocytes [16], does not increase the NCX mRNA (Fig. 3) and protein level (Fig. 4). This result suggests, that the observed up-regulation of the NCX is fairly specific and not due to a general anabolic response, i.e. increase of cellular protein. The difference of the NCX mRNA level between control and PE-treated ARC was more pronounced after 72 h of incubation (312% vs. 248% after 24 h compared to the 100% control). However, this effect was rather due to a decrease of NCX transcripts in the controls.

3.2. Western blot analysis

Western blot analysis using particular fraction protein of ARC treated with PE for 24 h revealed a specific immunoreactive band at ~ 120 kD as described by Philipson et al. [30] (Fig. 4A). PE-stimulated ARC showed a 1.8-fold increase of the NCX protein level as compared to controls ($p < 0.05$; $n = 5$). Consistent with the results described above, the increase of the NCX protein was

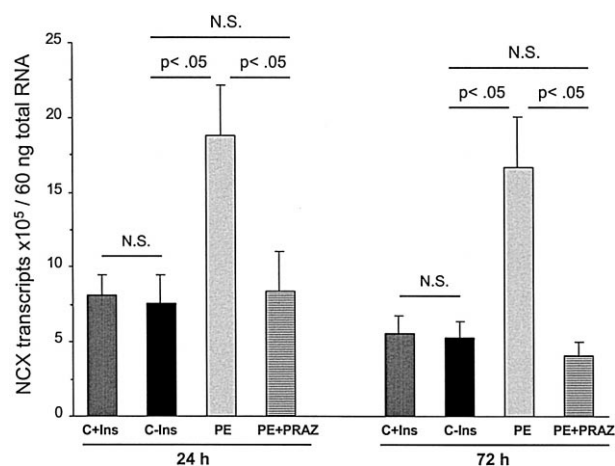


Fig. 3. Bar graphs displaying the results of competitive RT-PCR experiments. The number of NCX transcripts of ARC treated with phenylephrine (PE) for 24 h and 72 h was significantly increased. The effect of PE was blocked by the α_1 -adrenergic agonist prazosin (PE+PRAZ). Insulin had no effect on the NCX mRNA level (C+Ins vs. C-Ins).

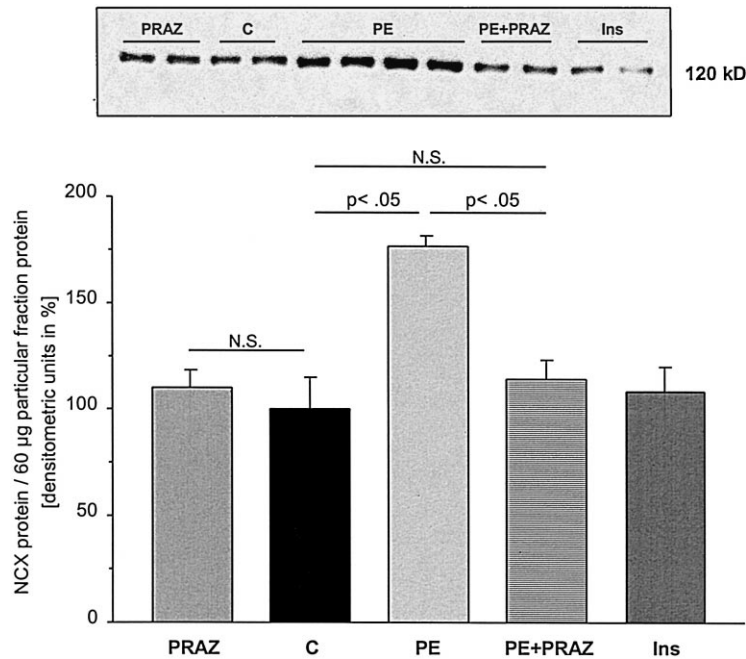


Fig. 4. (Top) Western blot analysis of the NCX in adult cardiocytes treated for 24 h with prazosin (PRAZ), phenylephrine (PE), phenylephrine + prazosin (PE + PRAZ), or insulin (Ins). Control = C. (Bottom) The amount of immunoreactive NCX protein was significantly increased in ARC treated for 24 h with PE. The PE effect was blocked by prazosin (PE + PRAZ). Insulin treatment had no effect on the NCX expression.

blocked by PRAZ ($p < 0.05$ vs. PE and N.S. vs. control) (Fig. 4B). No significant changes of the NCX protein level were found, when ARC were incubated with insulin or PRAZ (Fig. 4).

4. Discussion

Human heart failure has been shown to be accompanied and promoted by adrenoceptor stimulation and activation of the renin-angiotensin system leading to increased plasma levels of catecholamines [1,2]. Likewise, it is well established that myocardial Ca^{2+} -homeostasis is disturbed in experimental cardiac hypertrophy and in human heart failure [3]. In the present study, we used an adult rat cardiomyocyte cell culture system to investigate the effects of the α -adrenergic agonist phenylephrine, representing a well-known promoter of cardiac hypertrophy in vivo and in vitro [10], on the gene expression and function of the sarcolemmal NCX. The results demonstrate that activation of the α_1 -adrenoceptor induced a significant and prolonged up-regulation of the sarcolemmal NCX mRNA and protein level.

Recent studies using experimental models of pressure-overload induced cardiac hypertrophy in different species [6,7], have presented evidence for an enhanced expression and/or activity of the NCX due to increased mechanical load. In addition, studies from our laboratories have shown that the gene expression [8] as well as the activity [9] of the sarcolemmal NCX is increased in patients with severe heart failure. The hypothesis was raised that an increased

NCX expression and activity might, at least in part, compensate for a depressed SR function [8,9]. However, the benefit of limiting diastolic Ca^{2+} overload might be counteracted by the corresponding influx of Na^+ leading to membrane depolarization and enhanced arrhythmogenesis. It is well known that increased plasma levels of catecholamines are commonly found in patients with heart failure [1]. Therefore, the present findings might be of interest regarding the situation in man, too.

Evidence for an effect of α -adrenoceptor stimulation on the NCX gene expression was recently presented in a study of Menick and co-workers [31], showing that the mRNA level of the NCX is up-regulated by 3 to 5-fold in neonatal feline cardiocytes after only one hour of treatment with 100 μ M PE and remained up-regulated for at least four hours. We extend these findings considerably, showing that the effect of adrenergic stimulation on the NCX mRNA level is transferable to the adult cardiocyte maintained in serum-free culture and that this effect is sustained over 3 days. In addition, 24 h after stimulation we observed an increased protein level of the antiporter. It is well established that the gene expression pattern of the neonatal cardiomyocyte differs extensively from the adult phenotype [32]. For example, Ju and colleagues [34] have shown that angiotensin II treatment down-regulated mRNAs encoding Ca^{2+} transport genes (NCX, SR Ca^{2+} -ATPase and the SR Ca^{2+} -release channel) only in neonatal, but not in adult cardiocytes. The study strongly supports the notion that responses to applied stimuli can be extremely dependent on the developmental stage of cardiocytes. In particular, developmental changes were also

shown for the cardiac NCX in the rat heart [33], in which a high expression level of the NCX in the late fetal and early postnatal stage is followed by a steady decline during the development of the adult phenotype resulting in an 8-fold lower expression level compared to postnatal day one. Furthermore, cell cultures of neonatal cardiocytes generally imply the addition of serum, which might provide factors that are not present in the environment of the cardiocyte under physiological conditions. The present study shows that PE treatment for 24 h induced an increase of the NCX mRNA by 2.5-fold, of the protein by 1.8-fold. Regarding the increase of the NCX mRNA level, our result is fairly consistent with the findings of Menick et al. [31], although these data were raised in neonatal cardiocytes under different conditions.

Since α -adrenergic stimulation was shown to increase general protein synthesis in vivo and in vitro [10], it has to be considered whether the observed effects of PE on the NCX expression might be unspecific, i.e., reflecting a general increase of cellular protein synthesis. In control experiments, insulin (0.1 μ M) was added to the cell culture medium and insulin-treated ARC were incubated for 24 h and 72 h. Insulin is known to be a potent activator of general protein turn-over and, indeed, it has been shown that general protein synthesis in isolated adult rat cardiocytes is characteristically insulin-responsive [16]. Importantly, insulin incubation for 24 h and 72 h had no effect on the NCX gene expression, as measured by competitive RT-PCR (Fig. 3). This result strongly supports the idea of a specific α_1 -adrenoceptor mediated response leading to the up-regulation of the NCX. Furthermore, Menick and co-workers [31] also have shown that insulin (0.1 μ M) had no effect on the NCX mRNA level in adult cardiocytes.

We also performed Ca^{2+} transport measurements in PE-treated and untreated ARC according to a protocol described elsewhere [9,33]. However, Ca^{2+} uptake experiments failed for unknown reasons.

Kent and McDermott [35] have shown that passive load applied by step increments of stretch induced an up-regulation of the NCX mRNA. In conjunction with this study, at least two pathways seem to exist for the induction of the NCX up-regulation in the adult rat cardiocyte: load-dependent due to stretch of the cardiomyocyte and load-independent due to α -adrenergic stimulation. Load has been shown to alter the Na^+ and Ca^{2+} gradients across the sarcolemma [36,37]; α -adrenoceptor stimulation results in increased myoplasmic Ca^{2+} concentrations [21]. Evidence that changes of the myoplasmic ionic conditions may affect the NCX gene expression are supported by the fact that veratridine, a Na^+ and Ca^{2+} influx stimulator in excitable cells, induced an up-regulation of the NCX mRNA level, similar to load-dependent NCX up-regulation [35], and to the α -adrenoceptor mediated increase of NCX mRNA and protein, observed in the present study. Since cardiac hypertrophy and heart failure is closely associated with the activation of the adrenergic system and an up-regulation of

the cardiac NCX both in animal models as well as in man, future studies will address the pathophysiological relevance of the present findings in human heart failure.

Acknowledgements

We are grateful to Dr. K.D. Philipson for providing the guinea pig $\text{Na}^+/\text{Ca}^{2+}$ -exchanger cDNA. The excellent technical assistance of Robert Burzan and Christel Kemsis is greatly appreciated. This work was supported by grants from the Deutsche Forschungsgemeinschaft (Dr 148/6-1, Ve 136/1-3), H.D. is an Established Investigator of the German Research Foundation (DFG, Heisenberg-Stipendium Dr 148/5-2).

References

- [1] Parmley WW. Neuroendocrine changes in heart failure and their clinical relevance. *Clin Cardiol* 1995;18:440–445.
- [2] Parmley WW. Pathophysiology of congestive heart failure. *Clin Cardiol* 1992;15(Suppl.1):15–12.
- [3] Arai M, Matsui H, Periasamy M. Sarcoplasmic reticulum gene expression in cardiac hypertrophy and failure. *Circ Res* 1994;74:555–564.
- [4] Bers DM, Bridge JHB. Relaxation of rabbit ventricular muscle by Na - Ca exchange and sarcoplasmic reticulum calcium pump. *Circ Res* 1989;65:334–342.
- [5] Bers DM, Lederer WJ, Berlin JR. Intracellular Ca transients in rat cardiac myocytes: role of Na - Ca exchange in excitation-contraction coupling. *Am J Physiol* 1990;258:C944–C954.
- [6] Kent RL, Rozich JD, McCollam PL, McDermott DE, Thacker UF, Menick DR, McDermott PJ, Cooper G IV. Rapid expression of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger in response to cardiac pressure overload. *Am J Physiol* 1993;265:H1024–H1029.
- [7] Nakanishi H, Makino N, Hata T, Matsui H, Yano K, Yanaga T. Sarcolemmal Ca^{2+} transport activities in cardiac hypertrophy caused by pressure overload. *Am J Physiol* 1989;257:H349–H356.
- [8] Studer R, Reinecke H, Bilger J, Eschenhagen T, Böhm M, Hasenfuss G, Just H, Holtz J, Drexler H. Gene expression of the cardiac sodium-calcium exchanger in end-stage human heart failure. *Circ Res* 1994;75:443–453.
- [9] Reinecke H, Studer R, Vetter R, Holtz J, Drexler H. Cardiac $\text{Na}^+/\text{Ca}^{2+}$ exchange activity in patients with end-stage heart failure. *Cardiovasc Res* 1996;31:48–54.
- [10] Morgan HE, Baker KM. Cardiac hypertrophy: Mechanical, neural, and endocrine dependence. *Circulation* 1991;83:13–25.
- [11] Iwaki K, Sukhatme VP, Shubeita HE, Chien KR. α - and β -adrenergic stimulation induces distinct patterns of immediate early gene expression in neonatal rat myocardial cells. *fos/jun* expression is associated with sarcomere assembly; *Egr-1* induction is primarily an α_1 -mediated response. *J Biol Chem* 1990;265:13809–13817.
- [12] Starksen NF, Simpson PC, Bisphoric N, Coughlin SR, Lee WMF, Escobedo JA, Williams LT. Cardiac myocyte hypertrophy is associated with *c-myc* protooncogene expression. *Proc Natl Acad Sci USA* 1986;83:8348–8350.
- [13] Lee HR, Henderson AS, Reynolds R, Dunmon P, Yuan D, Chien KR. α_1 -Adrenergic stimulation of cardiac gene transcription in neonatal rat myocardial cells. *J Biol Chem* 1988;263:7352–7358.
- [14] Bisphoric NH, Simpson PC, Ordahl CP. Induction of skeletal α -actin gene in α_1 -adrenoceptor-mediated hypertrophy of rat cardiac myocytes. *J Clin Invest* 1987;80:1194–1199.

- [15] Simpson PC, Long CS, Waspe LE, Henrich CJ, Ordahl CP. Transcription of early developmental isogenes in cardiac myocyte hypertrophy. *J Mol Cell Cardiol* 1989;21(Suppl.5):79–89.
- [16] Fuller SJ, Gaitanaki CJ, Sugden PH. Effects of catecholamines on protein synthesis in cardiac myocytes and perfused hearts isolated from adult rat hearts. *Biochem J* 1990;266:727–736.
- [17] Ikeda U, Tsuruya Y, Yaginuma T. α_1 -Adrenergic stimulation is coupled to cardiac myocyte hypertrophy. *Am J Physiol* 1991;260:H953–H956.
- [18] Schlüter KD, Piper HM. Trophic effects of catecholamines and parathyroid hormone on adult ventricular cardiomyocytes. *Am J Physiol* 1992;263:H1739–H1746.
- [19] Henrick CJ, Simpson PC. Differential acute and chronic response of protein kinase C in cultured neonatal rat heart myocytes to α_1 -adrenergic and phorbol ester stimulation. *J Mol Cell Cardiol* 1988;20:1081–1085.
- [20] Nosek TM, Williams MF, Zeigler ST, Godt RE. Inositol trisphosphate enhances calcium release in skinned cardiac and skeletal muscle. *Am J Physiol* 1986;250:C807–C811.
- [21] Mann DL, Kent RL, Parsons B, Cooper G IV. Adrenergic effects on the biology of the mammalian cardiocyte. *Circulation* 1992;85:790–804.
- [22] Buxton ILO, Brunton LL. Alpha-adrenergic receptors on rat ventricular myocytes: characteristics and linkage to cAMP metabolism. *Am J Physiol* 1986;251:H307–H313.
- [23] Bode DC, Brunton LL. Adrenergic, cholinergic, and other hormone receptors on cardiac myocytes. In: Piper HM, Isenberg G, editors. *Isolated Adult Cardiomyocytes. Structure and Metabolism, Vol. I*. Boca Raton, FL: CRC, 1989: 163–202.
- [24] Powell T, Methods for the isolation and preparation of single adult myocytes. In: Clark WA, Decker RS, Borg TK, editors. *Biology of Adult Cardiac Myocytes*. Amsterdam: Elsevier, 1988: 9–13.
- [25] Rose H, Kammermeier H. Contraction and metabolic activity of electrically stimulated cardiac myocytes from adult rats. *Pflugers Arch* 1986;407:116–118.
- [26] Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. *Anal Biochem* 1987;162:156–159.
- [27] Studer R, Reinecke H, Müller B, Holtz J, Just H, Drexler H. Increased angiotensin-I converting enzyme gene expression in the failing human heart. *J Clin Invest* 1994;94:301–310.
- [28] Low W, Kasir J, Rahamimoff H. Cloning of the rat heart $\text{Na}^+ - \text{Ca}^{2+}$ -exchanger and its functional expression in HeLa cells. *FEBS Lett* 1993;318:63–67.
- [29] Tsuruya Y, Bersohn MM, Li Z, Nicoll DA, Philipson KD. Molecular cloning and functional expression of the guinea pig cardiac $\text{Na}^+ - \text{Ca}^{2+}$ -exchanger. *Biochem Biophys Acta* 1994;1196:97–99.
- [30] Philipson KD, Longoni S, Ward R. Purification of the cardiac $\text{Na}^+ - \text{Ca}^{2+}$ exchange protein. *Biochem Biophys Acta* 1988;945:298–306.
- [31] Menick DR, Barnes KV, Thacker UF, Dawson MM, McDermott DE, Rozich JD, Kent RL, Cooper IV, G. The exchanger and cardiac hypertrophy. In: Hilgemann DW, Philipson KD, Vassort G, editors. *Sodium-Calcium Exchange. Proceedings of the Third International Conference*. New York: Ann NY Acad Sci 1996;779:489–501.
- [32] Schwartz K, Mercadier J-J, Swynghedauw B, Lompré A-M. Modifications of the gene expression in cardiac hypertrophy. *Heart Failure* 1988;4:326–347.
- [33] Vetter R, Studer R, Reinecke H, Kolár, Ostádalová I, Drexler H. Reciprocal changes in the postnatal expression of the sarcolemmal $\text{Na}^+ - \text{Ca}^{2+}$ -exchanger and SERCA2 in rat heart. *J Mol Cell Cardiol* 1995;27:1689–1701.
- [34] Ju H, Scammel-La Fleur T, Dixon IM. Altered mRNA abundance of calcium transport genes in cardiac myocytes induced by angiotensin II. *J Mol Cell Cardiol* 1996;28:1119–1128.
- [35] Kent RL, McDermott PJ. Passive load and angiotensin II evoke differential responses of gene expression and protein synthesis in cardiac myocytes. *Circ Res* 1996;78:829–838.
- [36] Kent RL, Hooper JK, Cooper G IV. Load responsiveness of protein synthesis in adult mammalian myocardium: role of cardiac deformation linked to sodium influx. *Circ Res* 1989;64:74–85.
- [37] Haneda T, Watson PA, Morgan HE. Elevated aortic pressure, calcium uptake, and protein synthesis in rat heart. *J Mol Cell Cardiol* 1989;21(1):131–138.