ELSEVIER

European Journal of Pharmacology 422 (2001) 175–180

ejp

www.elsevier.nl/locate/ejphar

Nebivolol, carvedilol and metoprolol do not influence cardiac Ca^{2+} sensitivity

Andreas Bundkirchen, Klara Brixius, Birgit Bölck, Uwe Mehlhorn [1], Wilhelm Bloch [2], Robert H.G. Schwinger *

Labor für Herzmuskelphysiologie und Molekulare Kardiologie, Klinik III für Innere Medizin der Universität zu Köln, Joseph-Stelzmann-Strasse 9, D-50924 Cologne, (Lindenthal), Germany

Received 29 March 2001; received in revised form 9 May 2001; accepted 22 May 2001

Abstract

It has been argued that some β-adrenoceptor antagonists may directly influence myofibrillar cross-bridge interaction in cardiac skinned fiber preparations of animal models. The present study investigates the effects of nebivolol, metoprolol and carvedilol on tension development of Triton X-100 skinned fibers obtained from human failing myocardium as well as on force of contraction and intracellular Ca^{2+} transient in isolated trabeculae. In skinned fiber preparations, none of the β-adrenoceptor antagonists (10 μM) influenced Ca^{2+} sensitivity of tension development or maximal Ca^{2+} activated tension (DT_{max}): control: EC_{50} for Ca^{2+}: 1.28 ± 0.05 μM, DT_{max}: 14.09 ± 0.59 mN/mm^2; nebivolol: 1.36 ± 0.1 μM, 14.14 ± 0.95 mN/mm^2; carvedilol: 1.32 ± 0.11 μM, 13.83 ± 0.90 mN/mm^2; metoprolol: 1.34 ± 0.14 μM, 13.72 ± 0.36 mN/mm^2. Simultaneous measurement of force and Ca^{2+} transient in the presence of the β-adrenoceptor antagonists (3 μM) showed that the decrease in force of contraction was paralleled by a similar decrease in the intracellular Ca^{2+} transient. In conclusion, none of the investigated β-adrenoceptor antagonists influenced Ca^{2+} sensitivity of myofibrillar tension development in human failing myocardium.

Keywords: Ca^{2+} sensitivity; β-Adrenoceptor antagonist; Nebivolol; Heart failure; Myocardium, human

1. Introduction

In chronic heart failure patients, several β-adrenoceptor antagonists (e.g. carvedilol, metoprolol and bisoprolol) increase ventricular performance and decrease mortality when used on a long-term basis (Bristow, 2000; CIBIS II Investigators and Committees, 1999; MERIT-HF Study group, 1999; Packer et al., 1996). Acutely, however, β-adrenoceptor antagonists lower force development.

The underlying mechanisms of the beneficial effects of the β-adrenoceptor antagonists still remain unclear. Differences in several pharmacological properties (e.g. β_1/β_2-selectivity, additional vasodilating properties) should very likely go along with a different outcome in the clinical use of the β-adrenoceptor antagonists in heart failure patients. In addition, direct actions on Ca^{2+} activated cross-bridge formation (either stimulatory or inhibitory) could influence negative inotropic moiety of β-adrenoceptor antagonists.

In some animal models, it has been shown that β-adrenoceptor antagonists are able to influence myofibrillar cross-bridge interaction (Gwathmey et al., 1999; Kitada, 1996; Zeitz et al., 2000). In canine right ventricular skinned fibers, betaxolol and propranolol increased Ca^{2+} activated tension (Kitada, 1996), whereas in rabbit right ventricular skinned fiber preparation, propranolol had no influence on tension development in cardiac myofilaments (Zeitz et al., 2000). In cardiomyopathic turkey, carteolol restored myofibrillar ATPase activities (Gwathmey et al., 1999).

Influences in myofibrillar Ca^{2+} sensitivity might also be of importance in the human heart and thus for the treatment of heart failure patients. In human heart failure, diastolic Ca^{2+} is increased, systolic Ca^{2+} is significantly diminished and the decay of the intracellular Ca^{2+} transient is significantly prolonged as compared to control (Beuckelmann et al., 1992). Therefore, pharmacological agents which sensitize the myofilaments for Ca^{2+} may on the one hand increase systolic force development, on the

* Corresponding author. Tel.: +49-221-478-3138; fax: +49-221-478-3746.

E-mail address: Robert.Schwinger@medizin.uni-koeln.de (R.H.G. Schwinger).

[1] Klinik für Herz-und Thoraxchirurgie der Universität zu Köln, Germany.

[2] Institut I für Anatomie der Universität zu Köln, Germany.

0014-2999/01/$ - see front matter © 2001 Published by Elsevier Science B.V.
PII: S0014-2999(01)01082-2

other hand however, they may exaggerate the diastolic contractile dysfunction observed in human heart failure (Edes et al., 1995; Teramura and Yamakado, 1998; Ukkonen et al., 2000). Contrary, pharmacological agents which desensitize the myofilaments towards Ca^{2+} may contribute to a restoration of diastolic cardiac function but impair systolic contractility in heart failure.

The newly developed highly β_1-selective β-adrenoceptor antagonist nebivolol has been demonstrated to have only a weak impact on left ventricular performance (De Cree et al., 1992; Janssens, 1992). Thus, nebivolol may be beneficial for treating patients with impaired cardiac function. Consistently, nebivolol is actually tested in the SENIORS-trial in heart failure patients. In rabbit ventricular skinned fibers, nebivolol desensitized tension development (Zeitz et al., 2000). However, differences may exist in the cross-bridge interaction between species (Lues et al., 1988).

Therefore, the present study investigates the influence of nebivolol, carvedilol and metoprolol on myofibrillar Ca^{2+} sensitivity as well as on force of contraction and the intracellular Ca^{2+} transient in human myocardial tissue.

2. Materials and methods

2.1. Tissue

Left ventricular tissue was obtained during cardiac transplantation due to dilated cardiomyopathy ($n = 3$; one female, two males; age: 61.3 ± 3.5 years; ejection fraction: 22.7 ± 1.8; cardiac index: 2.2 ± 0.1 l/m^2 min). Patients suffered from heart failure clinically classified as New York Heart Association Class (NYHA) IV on the basis of clinical symptoms and signs as judged by the attending cardiologist shortly before operation. Medical therapy consisted of diuretics, nitrates, angiotensin-converting enzyme inhibitors and cardiac glycosides. Patients receiving catecholamines, β-adrenoceptor or Ca^{2+} antagonists were withdrawn from the study.

Right atrial tissue was taken from patients undergoing aortocoronary bypass operation ($n = 6$; two females, four males; age: 69.0 ± 4.7 years) without clinical signs of cardiac failure as measured by heart catheterization (normal ejection fraction, enddiastolic volume, and stroke volume) and by echocardiography. None of the patients has received Ca^{2+} channel antagonists or Ca^{2+} channel agonists within 7 days of surgery, or β-adrenoceptor agonists 48 h before surgery.

All patients gave written informed consent before surgery. Drugs used for general anesthesia were propofol, flunitrazepam, fentanyl, and pancuronium bromide.

The tissue was delivered within 5 min into the laboratory in ice-cold preaerated Bretschneider solution of the following composition (in mM): NaCl 15, KCl 10 $MgCl_2$ 4, histidine 180, tryptophane 2, mannitol 30, and potassium dihydrogen oxoglutarate 1. From each native right atrial tissue sample, auricular trabeculae were selected of 0.4–0.6 mm width and 5–7 mm length under microscopic control (Axiovert 100, Carl Zeiss, Oberkochen, Germany).

2.2. Simultaneous measurement of force and intracellular Ca^{2+} transient

Intracellular Ca^{2+} was measured in isolated, electrically driven (30 to 180 bpm, punctuate stimulation, 1 Hz, 37 °C) trabeculae by the fluorescence indicator fura-2 acetoxy methylester (fura-2 AM) (Grynkiewicz et al., 1985). Experiments were performed as described previously (Brixius et al., 1997).

2.3. Measurement of myofibrillar Ca^{2+} sensitivity

Triton X-100 skinned fibers were prepared as described previously (Schwinger et al., 1994). Fiber diameter and length were the same in all preparations studied (125 to 175 μm and 7 to 8 mm, respectively). The mean sarcomere length of the skinned fibers was 2.1 ± 0.1 μm in relaxation solution as measured by α-actinin staining (Brixius et al., 2000; Ji et al., 1999). The relaxation solution contained (in mM) imidazol buffer 40, ATP 10, $MgCl_2$ 12.5, creatine phosphate 10, NaN_3 5, EGTA 5, and Dithioerythritol (DTE) 1, along with 350 U/ml (pH 7.0) creatine kinase. The contraction solution had the same composition as the relaxation solution except that $CaCl_2$ was added. The desired $[Ca^{2+}]$ was obtained by mixing the relaxation and contraction solution in the appropriate concentrations. The contraction solution had a $[Ca^{2+}]$ of 16.52 μM. All experiments were performed at 21 °C. The actual $[Ca^{2+}]$ values were calculated by a computer program according to that reported by Fabiato and Fabiato (1979). From each heart, several skinned-fiber preparations of ventricle were investigated. Concentration–response curves for Ca^{2+} were measured in the presence and in the absence of nebivolol, carvedilol or metoprolol (all 10 μM). In all experiments, the final concentration of solvent (dimethyl sulfoxide, DMSO) was 0.1%. EC_{50}-concentration for Ca^{2+} was calculated for each fiber. Experiments were performed as described previously (Schwinger et al., 1994).

2.4. Materials

Nebivolol was generously provided by Berlin Chemie (Berlin, Germany), carvedilol was provided by Boehringer (Mannheim, Germany) and metoprolol was a gift from Astra (Wedel, Germany). Triton X-100 was from Merck (Darmstadt, Germany).

Fura-2 AM was obtained from Molecular Probes (Eugene, OR, USA). A stock solution of fura-2 AM (10 mM) was dissolved in DMSO and stored at −20 °C. For studies with isolated cardiac preparations, stock solutions were daily prepared in twice-distilled water. All other chemicals were of analytical grade or the best grade commercially available.

2.5. Statistics

All values are means ± S.E.M. unless otherwise noted. Student's *t*-test, or paired *t*-test were used to test significance. Significance was accepted at a *P*-value of < 0.05.

For each experiment, IC_{50} and EC_{50} values, respectively were calculated by regression analyses (GraphPad Software, San Diego, USA).

3. Results

3.1. Influence on the contractile apparatus

To investigate the influence of nebivolol, carvedilol or metoprolol on cross-bridge interaction, Ca^{2+}-dependent tension development was studied in Triton X-100 skinned fiber preparations of human left ventricular myocardium.

Fig. 1 shows a representative original recording of Ca^{2+} activated tension development. Tension was Ca^{2+} dependently increased in these preparations. Maximal Ca^{2+} activated tension was achieved at a free Ca^{2+} concentration of 16.52 μM. Nebivolol, carvedilol and metoprolol (all 10 μM) did not influence maximal Ca^{2+} activated tension (control: DT_{max}: 14.09 ± 0.59 mN/mm^2, 95% confidence interval: 12.83–15.35 mN/mm^2, $n = 15$; nebivolol: 14.14 ± 0.95 mN/mm^2, 11.80–16.48 mN/mm^2, $n = 7$; carvedilol: 13.83 ± 0.90 mN/mm^2, 11.51–16.14 mN/mm^2, $n = 6$; metoprolol: 13.72 ± 0.36 mN/mm^2, 12.88–14.57 mN/mm^2, $n = 8$).

In addition, no shift was observed for the Ca^{2+}-dependent tension development in the presence of nebivolol, carvedilol or metoprolol (10 μM) (Fig. 2). The concentrations needed for a 50% increase of maximal Ca^{2+} activated tension (EC_{50}) were: control: EC_{50}: 1.28 ± 0.05 μM, 95% confidence interval: 1.19–1.39 μM; nebivolol: 1.36 ± 0.1 μM, 1.10–1.61 μM; carvedilol: 1.32 ± 0.11 μM, 1.04–1.60 μM and metoprolol: 1.34 ± 0.14 μM, 1.00–1.68 μM, respectively.

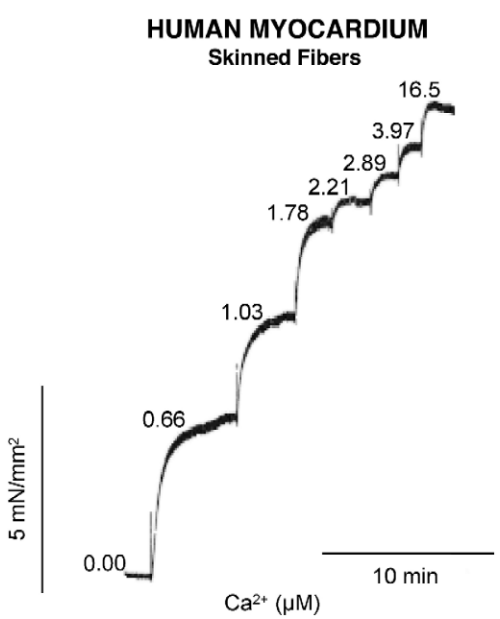

Fig. 1. Original tracing recording (control fiber) illustrating measurement of Ca^{2+}-dependent tension development in Triton X-100 skinned fibers from human failing myocardium.

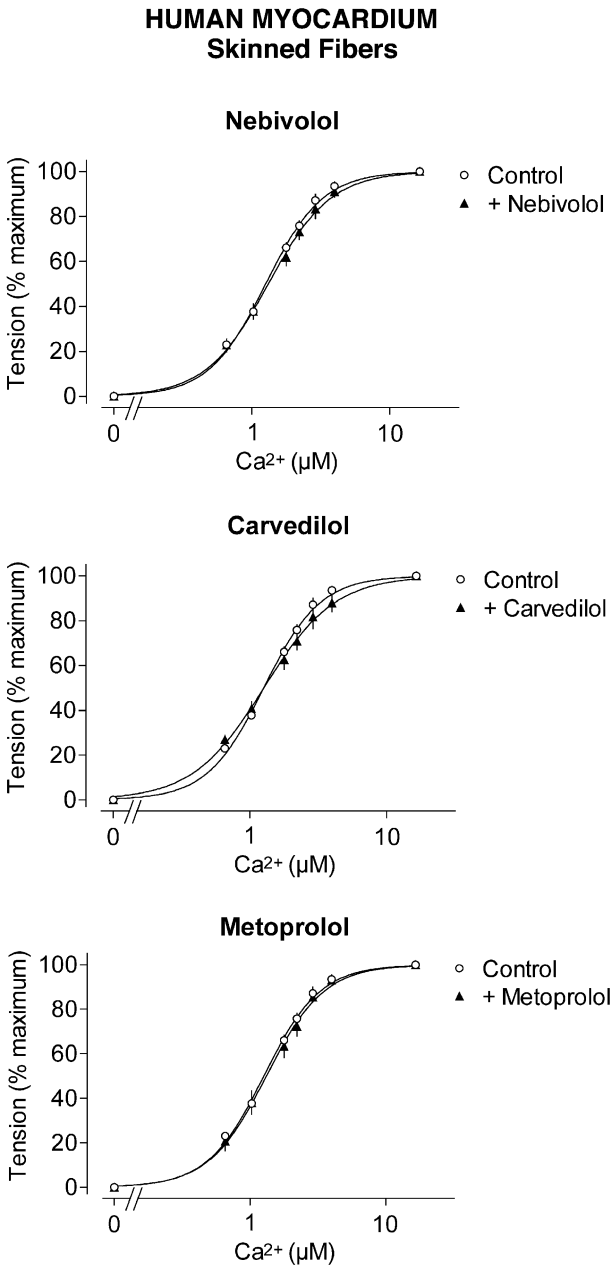

Fig. 2. Influence of β-adrenoceptor antagonists (10 μM) on Ca^{2+} sensitivity measured in Triton X-100 skinned fiber preparations of human left ventricular failing myocardium. Note that neither nebivolol nor carvedilol or metoprolol influenced Ca^{2+} sensitivity.

3.2. Influence on force and Ca^{2+} transient

Besides direct cross-bridge interaction, Ca^{2+} sensitization or Ca^{2+} desensitization could be due to signal interac-

tions on the way between the cell membrane and the cardiac myofibrillars. Therefore, we performed simultaneous measurements of force of contraction and the intracellular Ca^{2+} transient in isolated right auricular trabeculae.

Fig. 3 shows original recording under baseline conditions and after application of nebivolol, carvedilol and metoprolol, respectively (all 3 μM). The simultaneous measurements were recorded under baseline conditions and after application of nebivolol, carvedilol or metoprolol (3 μM). Each β-adrenoceptor antagonist induced a decrease in force of contraction accompanied by a similar decrease in the intracellular Ca^{2+} transient (Fig. 4). Basal force of contraction and basal Ca^{2+} transient was similar in all groups (nebivolol: force of contraction: 10.32 ± 0.67 mN/mm^2, Ca^{2+} transient: 0.16 ± 0.02, $n = 4$; carvedilol: force of contraction: 11.68 ± 2.52 mN/mm^2, Ca^{2+} transient: 0.23 ± 0.09, $n = 4$; metoprolol: force of contraction: 14.07 ± 2.22 mN/mm^2, Ca^{2+} transient: 0.24 ± 0.06, $n = 4$). The rank order of lowering force of contraction and Ca^{2+} transient at 3 μM was metoprolol (force of contraction: $-45.0 \pm 5.0\%$; Ca^{2+} transient: $-27.0 \pm 5.4\%$) > carvedilol ($-20.5 \pm 7.5\%$; $-8.7 \pm 1.9\%$) > nebivolol ($-12.5 \pm 5.7\%$; $-8.3 \pm 3.5\%$). Control experiments showed no significant decrease in force of contraction and Ca^{2+} transient for the time of pharmacological testing. These results indicate that the decrease in force of contraction induced by nebivolol, carvedilol and metoprolol is paralleled by a decrease of the intracellular Ca^{2+} transient.

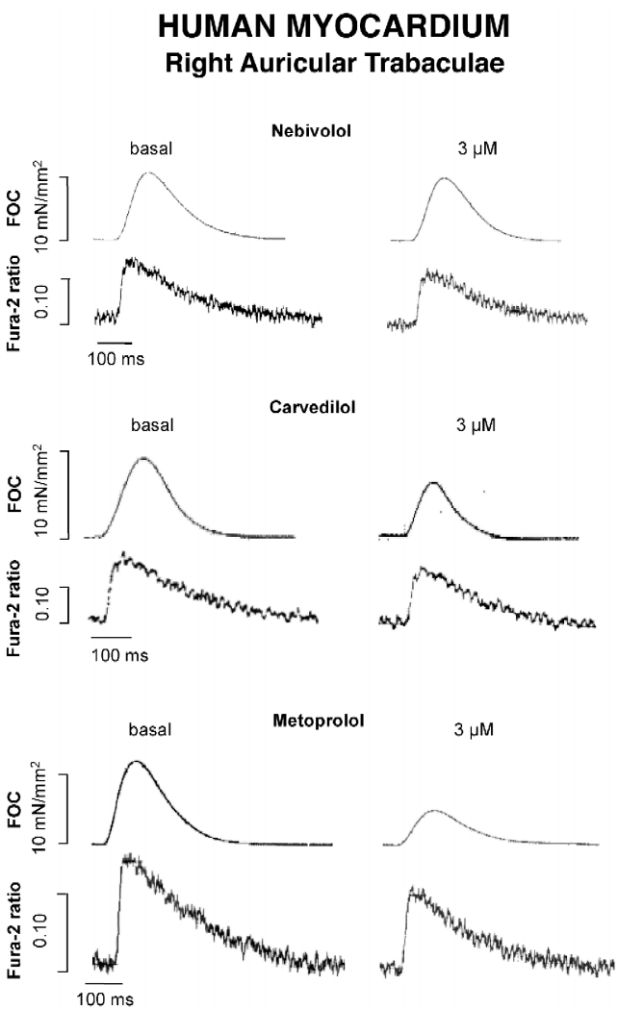

Fig. 3. Original tracings illustrating the effect of nebivolol, carvedilol and metoprolol on force of contraction and Ca^{2+} transient in isolated, electrically driven (1 Hz) auricular trabeculae of human myocardium.

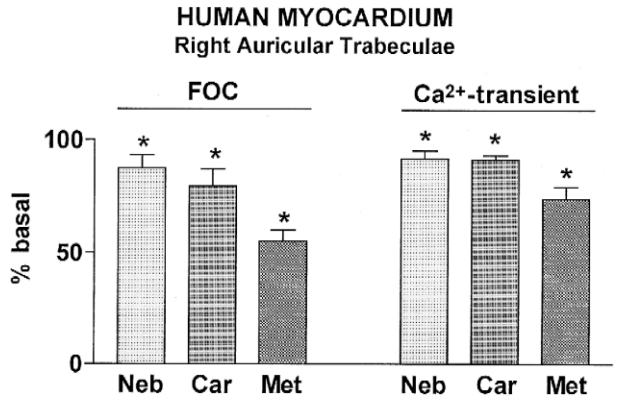

Fig. 4. Effect of nebivolol (Neb), carvedilol (Car) and metoprolol (Met) on force of contraction and Ca^{2+} transient. $^{*}P < 0.05$ vs. basal.

4. Discussion

In congestive heart failure patients, the application of β-adrenoceptor antagonists decreases mortality (Bristow, 2000; CIBIS II Investigators and Committees, 1999; MERIT-HF Study group, 1999; Packer et al., 1996). However, the mechanism by which the β-adrenoceptor antagonists mediate their beneficial effects in heart failure patients are not very well understood. The finding that not all of the β-adrenoceptor antagonists have beneficial effects in heart failure (Nicholas et al., 1990) underlines the necessity to further investigate the cardiac effects of the different β-adrenoceptor antagonists on the molecular level.

In some animal models, it has been demonstrated that some of the β-adrenoceptor antagonists might have an impact on myofibrillar Ca^{2+} sensitivity (Gwathmey et al., 1999; Kitada, 1996; Zeitz et al., 2000). In canine right ventricular skinned fibers, betaxolol and propranolol increased Ca^{2+} activated tension (Kitada, 1996), whereas in rabbit right ventricular skinned fiber preparation, propranolol had no influence on sensitivity of cardiac myofilaments (Zeitz et al., 2000). The findings are, at least partially contradictory but might be explained by interspecies differences or experimental setups. These interspecies- or setup-dependent differences hold true for skinned fiber experiments investigating other drugs (Edes et al., 1995; Lues et al., 1988). Influences on the contractile apparatus should, of course, be of importance for

myocardial contractility of patients treated with β-adrenoceptor antagonists. Thus, we aimed to study if the β-adrenoceptor antagonists nebivolol, carvedilol and metoprolol influence Ca^{2+} sensitivity in human myocardium. Neither nebivolol nor the very well-characterized carvedilol or metoprolol had an impact on myofibrillar Ca^{2+} sensitivity in human Triton X-100 skinned fibers. These results are different from measurements of nebivolol in rabbit ventricular skinned fibers (Zeitz et al., 2000). This may be due to interspecies differences. However, mechanisms on the way between the cell membrane and the myofilaments may exist, which cannot be determined by measurements in Triton X-100 skinned fiber preparations, but may have an influence on Ca^{2+} sensitivity of the myofilaments. Simultaneous measurement of force of contraction and the intracellular Ca^{2+} transient in isolated trabeculae showed that the decrease in force of contraction induced by the β-adrenoceptor antagonists was accompanied by a similar percentage decrease in Ca^{2+} transient, which is indicative that Ca^{2+} desensitizing mechanisms cannot be of relevance for the negative inotropic effects mediated by the β-adrenoceptor antagonists.

4.1. Limitation of the study

The present study was performed under in vitro conditions. It cannot be excluded that the in vivo effects of the studied β-adrenoceptor antagonists may differ from those observed in vitro (e.g. in vivo, varying sympathoadrenergic stimulation is present which might have an impact on β-adrenoceptor interaction).

In addition, experiments were either performed on isolated right auricular trabeculae from human non-failing hearts or on skinned fiber preparations of left ventricular failing myocardium. It cannot be excluded that a difference between failing and non-failing myocardium or between right atrial and left ventricular myocardium, respectively, may be of importance for the effect of β-adrenoceptor antagonists on myofibrillar Ca^{2+} sensitivity.

In our experimental setup, Ca^{2+} sensitizing or Ca^{2+} desensitizing mechanisms of β-adrenoceptor antagonists were investigated at concentrations that exceed their affinity to β_1- and β_2-adrenoceptors. In addition, the affinities of nebivolol, carvedilol and metoprolol to β_1- and β_2-adrenoceptors are different. However, Ca^{2+} sensitizing or Ca^{2+} desensitizing mechanisms should be independent from their affinity to β_1- and β_2-adrenoceptors and such additional properties should be rather unmasked at a high concentration of the β-adrenoceptor antagonists.

Acknowledgements

We are indebted to all our colleagues in the Department of the Cardiothoracic Surgery of the University of Cologne (Director: Prof. Dr. R.E. de Vivie) and Munich (Director: Prof. Dr. B. Reichart) for providing us with human myocardial samples. We also want to thank Sabine Danneschewski, Sabine Pfeiffer and Katja Rösler for their excellent technical help. This paper contains part of the doctor thesis of A.B. This work was supported by a grant of Köln Fortune (to K.B., R.H.G.S.) and DFG (R.H.G.S.).

References

Beuckelmann, D.J., Näbauer, M., Erdmann, E., 1992. Intracellular calcium handling in isolated ventricular myocytes from patients with terminal heart failure. Circulation 85, 1046–1055.

Bristow, M.R., 2000. β-Adrenergic receptor blockade in chronic heart failure. Circulation 101, 558–569.

Brixius, K., Pietsch, M., Hoischen, S., Müller-Ehmsen, J., Schwinger, R.H.G., 1997. Effect of inotropic interventions on contraction and Ca^{2+} transients in the human heart. J. Appl. Physiol. 83, 652–660.

Brixius, K., Mehlhorn, U., Bloch, W., Schwinger, R.H.G., 2000. Different effect of the Ca^{2+} sensitizers EMD 57033 and CGP 48506 on cross-bridge cycling in human myocardium. J. Pharmacol. Exp. Ther. 295, 1284–1290.

CIBIS II Investigators and Committees, 1999. The cardiac insufficiency bisoprolol Study II (CIBIS II): a randomized trial. Lancet 353, 9–13.

De Cree, J., Van Nueten, L., Geukens, H., Verhaegen, H., 1992. Comparative cardiac haemodynamics of bisoprolol, celiprolol, carvedilol and nebivolol in normal volunteers. Int. J. Clin. Pharmacol. Res. 12, 159–163.

Edes, I., Kiss, E., Kitada, Y., Powers, F.M., Papp, J.G., Kranias, E.G., Solaro, R.J., 1995. Effects of levosimendan, a cardiotonic agent targeted to troponin C, on cardiac function and on phosphorylation and Ca^{2+} sensitivity of cardiac myofibrils and sarcoplasmic reticulum in guinea pig heart. Circ. Res. 77, 107–113.

Fabiato, A., Fabiato, F., 1979. Calculator programs for computing the composition of the solutions containing multiple metals and ligands used for experiments in skinned muscle cells. J. Physiol. (Paris) 75, 463–505.

Grynkiewicz, G., Poenie, M., Tsien, R.Y., 1985. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. J. Biol. Chem. 260, 3440–3450.

Gwathmey, J.K., Kim, C.S., Hajjar, R.J., Khan, F., DiSalvo, T.G., Matsumori, A., Bristow, M.R., 1999. Cellular and molecular remodeling in a heart failure model treated with the β-blocker carteolol. Am. J. Physiol. 276, H1678–H1690.

Janssens, W.J., 1992. Pharmacology of nebivolol. J. Pharm. Belg. 47, 323–327.

Ji, G.J., Fleischmann, B.K., Block, W., Feelisch, M., Andressen, C., Addicks, K., Hescheler, J., 1999. Regulation of the L-type Ca^{2+} channel during cardiomyogenesis: switch from NO to adenylyl cyclase-mediated inhibition. FASEB J. 13, 313–324.

Kitada, Y., 1996. Contrasting effects of betaxolol and propranolol on Ca^{2+}-activated contractions in skinned fibers from canine coronary arteries and ventricular muscles. Cardiovasc. Drugs Ther. 10, 581–586.

Lues, I., Siegel, R., Harting, J., 1988. Effect of isomazole on the responsiveness to calcium of the contractile elements in skinned cardiac muscle fibres of various species. Eur. J. Pharmacol. 146, 145–153.

MERIT-HF Study Group, 1999. Effect of metoprolol CR/XL in chronic heart failure: metoprolol CR/XL randomised intervention trial in congestive heart failure. Lancet 353, 2001–2007.

Nicholas, G., Oakley, C., Pouleur, H., Rousseau, M.F., Rydén, l.E., Wellens, H., 1990. For the xamoterol in heart failure study group. Lancet 336, 1–6.

U.S. Carvedilol Heart Failure Study Group, Packer, M., Bristow, M.R., Cohn, J.N., Colucci, W., Fowler, M.B., Gilbert, E.M., Shusterman,

N.H., 1996. The effect of carvedilol on morbidity and mortality in patients with chronic heart failure. N. Engl. J. Med. 334, 1349–1355.

Schwinger, R.H.G., Böhm, M., Koch, A., Schmidt, U., Morano, I., Eissler, H.J., Überfuhr, P., Reichert, B., Erdmann, E., 1994. The failing human heart is unable to use the Frank–Starling mechanism. Circ. Res. 74, 959–969.

Teramura, S., Yamakado, T., 1998. Calcium sensitizers in chronic heart failure: inotropic interventions–reservation to preservation. Cardiologia 43, 375–385.

Ukkonen, H., Saraste, M., Akkila, J., Knuuti, J., Karanko, M., Iida, H., Lehikoinen, P., Nagren, K., Lehtonen, L., Voipio-Pulkki, L.M., 2000. Myocardial efficiency during levosimendan infusion in congestive heart failure. Clin. Pharmacol. Ther. 68, 522–531.

Zeitz, O., Rahman, A., Hasenfuss, G., Janssen, P.M., 2000. Impact of β-adrenoceptor antagonists on myofilament calcium sensitivity of rabbit and human myocardium. J. Cardiovasc. Pharmacol. 36, 126–131.