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Cardiovascular effects of C-type natriuretic peptide (CNP) overexpression in transgenic animals

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Kardiovaskuläre Effekte der Überexpression von C-Typ natriuretischem Peptid in transgenen Tieren

Yong Wang

Zusammenfassung

Die Familie der natriuretischen Peptide besteht aus drei strukturverwandten Peptiden, atriales (ANP), B-Typ (BNP) und C-Typ (CNP) natriuretischen Peptid. Die natriuretischen Peptide spielen eine wichtige Rolle in der kardiovaskulären Regulation unter pathophysiologischen Bedingungen. Im Rahmen meiner Arbeit habe ich zum einen Effekte der Kardiomyozyten-spezifischen CNP-Überexpression auf Ischämie/Reperfusion (I/R) und nach Myokardinfarkt (MI) in transgenen Mäusen untersucht. Des Weiteren habe ich die Bedeutung der drei Peptide in Diagnose und Prognose bei Patienten mit Chagas-Erkrankung (CD) und anderen dilatativen Kardiomyopathien (DCM) untersucht.

Es wurden transgene Mäuse mit Kardiomyozyten-spezifischer CNP-Überexpression hergestellt. Die erhöhte CNP-Expression wurde auf RNA- und Protein-Ebene mit „RNase-protection assay“ bzw. „Radioimmunoassay“ (RIA) nachgewiesen. Männliche transgene Mäuse und gleichaltrige Wildtypen wurden einer I/R (1h/23h) oder einer 3-wöchigen, permanenten Ligatur der Koronararterie unterzogen. Es wurden Blutproben von Patienten mit CD oder DCM und gleichaltrigen Gesunden genommen und die Konzentration der natriuretischen Peptide mittels RIA bestimmt.

Es gab keine Unterschiede in der Infarktgröße zwischen transgenen Tieren und Kontrollen bei den I/R Experimenten. Im Gegensatz dazu verhinderte die CNP Überexpression sowohl linksventrikuläre, als auch rechtsventrikuläre Hypertrophie drei Wochen nach MI-Induktion. Die histologische Untersuchung zeigte eine geringere Muskeldegeneration und Entzündung im Vergleich zu den infarzierten Wildtypen. Nekrose und Beeinträchtigung der Herzfunktion war in transgenen Tieren weniger stark ausgeprägt, als in den Kontrollen. Sowohl ANP-, als auch BNP-Spiegel waren in CD- und DCM-Patienten in Abhängigkeit von der NYHA-Klassifizierung erhöht. Eine signifikante Erhöhung war sogar in CD-Patienten ohne systolische ventrikuläre Dysfunktion zu sehen. Beide Peptidspiegel eignen sich gleichermaßen, um die Sterblichkeit oder die Notwendigkeit einer Herztransplantation zu prognostizieren, wohingegen CNP-Spiegel hierfür ungeeignet sind.

Abschließend lässt sich festhalten, dass die Überexpression von CNP in Kardiomyozyten keinen Einfluss auf die I/R-induzierte Infarktgröße hat, jedoch der MI-bedingten Hypertrophie

vorbeugt. Folglich könnte CNP eine therapeutische Option für die Behandlung von Patienten mit kardialer Hypertrophie, verursacht durch MI oder auf Grund anderer Ätiologie, sein. Während ANP und BNP gute Prognosemarker für die Sterblichkeit bei CD und DCM darstellen, zeigt CNP keine prognostische Potenz.

Cardiovascular effects of C-type natriuretic peptide (CNP) overexpression in transgenic animals

Yong Wang

Abstract

The natriuretic peptide family consist of three structurally related peptides, i.e., atrial (ANP), brain (BNP), and C-type (CNP) natriuretic peptide, which play an important role in cardiovascular regulation under pathophysiological conditions. The aim of our study was to investigate the effects of cardiomyocyte-specific CNP overexpression on ischaemia/reperfusion (I/R) injury and myocardial infarction (MI) in transgenic mice and to characterize diagnostic and prognostic properties of the three peptides in patients with Chagas' disease (CD) or other dilated cardiomyopathies (DCM).

Transgenic (TG) mice over-expressing CNP in cardiomyocytes were generated. Elevated CNP expression on RNA and protein levels was demonstrated by RNase-protection assay and radioimmunoassay. Male TG mice and age-matched wild-type (WT) littermates were subjected to I/R or permanent ligation of the coronary artery for three weeks. Blood samples from patients with CD, DCM or gender- and age-matched healthy subjects were obtained and peptide concentrations were determined by radioimmunoassays.

Infarct size did not differ between the WT and TG groups in mice subjected to I/R. In mice that underwent permanent ligation of coronary arteries, both left and right ventricular hypertrophy were prevented by CNP overexpression three weeks post MI. Histological analysis revealed less muscular degeneration and inflammation in TG mice three weeks post MI. Necrosis and impairment of cardiac function were less pronounced in transgenic animals than in the wild-type controls. Both ANP and BNP levels were increased in CD and DCM patients in relation to the NYHA class, even significantly elevated in CD patients without systolic ventricular dysfunction, and had comparable ability to predict death or the necessity for heart transplantation. However, CNP level could not predict mortality or the necessity for heart transplantation.

In conclusion, overexpression of CNP in cardiomyocytes does not affect I/R-induced infarct size but prevents cardiac hypertrophy induced by MI. Therefore, CNP may represent a therapeutic target for the treatment of patients with cardiac hypertrophy induced by myocardial infarction or other aetiology. While ANP and BNP have a high predictive value for mortality in both diseases, CNP is without any predictive potency.

Introduction

Natriuretic peptide family consists of three structurally related peptides: atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP). In contrast to ANP and BNP, which are produced mainly in cardiac atria and ventricles, respectively, CNP occurs in a wide variety of tissues (1), and is commonly considered to be an endothelial hormone. The biological actions of natriuretic peptides are mediated by three different membrane-bound receptor subtypes, natriuretic peptide receptor A, B, and C (NPRA, NPRB and NPRC) (2). NPRA and NPRB are guanylyl cyclase (GC)-coupled receptors, which can convert guanosine triphosphate (GTP) to guanosine 3',5'-cyclic monophosphate (cGMP). NPRC is a single transmembrane receptor with a short 37-amino-acid intracellular tail that lacks GC activity. ANP and BNP are ligands of NPRA, while CNP preferentially binds to NPRB. NPRC has similar affinity to all three natriuretic peptides and is commonly considered a clearance receptor.

Accumulating evidence suggests that CNP exhibits important autocrine and paracrine functions within the heart and coronary circulation (3). Hobbs *et al.* demonstrated that endothelium-derived CNP is involved in the regulation of rat coronary circulation via the activation of NPRC (4). They further showed that this newly defined CNP/NPRC pathway represents a protective mechanism against ischemia/reperfusion (I/R) injury in isolated perfused hearts, since infusion of CNP resulted in a 30-50% reduction in the infarct size (4). Furthermore, *in vivo* administration of CNP has been shown to improve cardiac function and attenuate cardiac remodeling after myocardial infarction in rats (5).

Chagas' disease (CD) caused by the protozoan *Trypanosoma cruzi* remains a leading cause of heart disease in Latin America. Evidences from human and animal studies suggest that chronic Chagasic cardiomyopathy is a complex disease resulting from progressive pathophysiological processes developing years after infection.

Objectives

My research aimed to investigate the functional impact of CNP overexpression in cardiomyocytes on infarct size and cardiac remodelling induced by myocardial I/R injury or myocardial infarction and to characterize and compare diagnostic and prognostic potency of the three natriuretic peptides in plasma of patients with CD and other dilated cardiomyopathies (DCM).

Methods

1. Generation of transgenic mice

Generation of transgenic mice specifically overexpressing CNP (RNCNP) in cardiomyocytes was done in core facilities of the Max-Delbrück-Center in Berlin-Buch prior to the start of this thesis. Different lines were detected by Southern blot.

2. Genotyping of transgenic mice

Primers in the αMHC promoter region and the CNP gene were used to perform PCR to detect the presence of the CNP transgene in mice genomic DNA isolated from tail biopsies.

3. RNase protection assay (RPA)

Total RNA was isolated from tissues using the TRIzol reagent (Invitrogen GmbH, Karlsruhe, Germany). RNCNP, mouse BNP and collagen type III mRNA expression were identified by RPA using the Ambion RPA II kit (Ambion (Europe) Ltd., Huntingdon, U.K.). The hybridized fragments protected from RNase A + T1 digestion were separated by electrophoresis on a denaturing gel and analyzed using a FUJIX BAS 2000 Phospho-Imager system (Raytest GmbH, Straubenhardt, Germany). Quantitative analyses were performed by measuring the intensity of the target bands normalized by the intensity of rL32.

4. Bioassay for CNP

After weighing organs, seven volumes of ice-cold 0.5 N acetic acid was added and homogenized by a T8 homogenizer (IKA GmbH, Staufen, Germany). The supernatants or plasma were extracted using Sep-Pak C_{18} cartridges, and the eluates were lyophilized and dissolved in an assay buffer of a commercial radioimmunoassay kit for CNP (Immundiagnostik AG, Bensheim, Germany). CNP concentration was normalized by total protein concentration and expressed as pg/mg for organs and pg/ml for plasma.

5. cGMP measurement

Levels of cGMP in ventricles and plasma were measured using a commercially available low-pH cGMP immunoassay kit (R&D Systems, Minneapolis, MN, USA). cGMP concentration was expressed as pmol/mg for organs and pmol/ml for plasma.

6. Animal experimental protocols

6.1. Blood pressure measurement

Mice were sedated with 4% isoflurane, intubated and artificially ventilated with a mixture of 70% room air and 30% oxygen including 2% isoflurane for anesthesia. A PE10 cannula was

inserted into the left carotid artery to monitor systolic blood pressure (SBP), mean arterial pressure (MAP), diastolic blood pressure (DBP) and heart rate (HR) for 15 min using BMON software (TSE GmbH, Bad Homburg, Germany).

6.2. Cardiac surgeries

Before starting instrumentation, the mice received 0.05 mg/kg analgesics (buprenorphine hydrochloride) subcutaneously, which was repeated once 12 hours after surgery. Surgical techniques were employed according to Tarnavski *et al.* (6) with some minor revisions. Regional I/R was produced by one-hour occlusion of the left anterior descending coronary artery (LAD) with a sterile 7.0 silk suture followed by 23 hours of reperfusion. Mice were then sacrificed and myocardial infarct size was measured.

In another group of mice, MI was produced by permanent ligation of the LAD. Echocardiographic and hemodynamic measurements were performed three weeks after MI, in which RV, LV and wet lung weights were obtained. One half of the LV was snap frozen in liquid nitrogen, and the other half was fixed in 4% paraformaldehyde for histological analysis.

6.3. Echocardiographic and hemodynamic analysis

Three weeks after MI, M-mode echocardiograms of the LV (ProSound SSD-4000, Aloka, Tokyo, Japan) were obtained using a 13-MHz probe. LV diameters were measured at end diastole (LV_{EDD}) and end systole (LV_{ESD}), and fractional shortening was calculated. After echocardiography, a polyethylene catheter (PE-10) was inserted into the left carotid artery to measure MAP. A 1.4-F-microtipped pressure transducer catheter (Millar Instruments, Houston, TX, USA) was inserted into the LV lumen via the right carotid artery to measure LV pressure. Subsequently, baseline recordings were obtained for MAP, HR and LV systolic pressure. In addition, we measured the contractility parameter $LVdP/dt_{max}$, the afterload independent $LVdP/dt_{P30}$ (positive LVdP/dt at LV pressure of 30 mmHg) and the relaxation parameters Tau (τ) and $LVdP/dt_{min}$.

7. Measurement of infarct size

After reperfusion, the ligature around the LAD was retied and 1 ml of 1% Evan's blue dye was injected into the jugular vein to delineate the area at risk (AAR). The heart was quickly excised, frozen for a few minutes at −20°C, and then immediately sliced with a scalpel into 1-mm-thick sections perpendicular to the long axis of the heart. Slices were incubated individually in 2% triphenyltetrazolium chloride (TTC) (Fluka, Buchs, Switzerland) in Sörensen buffer (pH 7.4) at 37°C for 5 min. Thereafter, the RV was removed, and each slice

of LV was weighed and photographed on both sides. The infarct size was expressed as a percentage of infarct area (IA) over total AAR.

8. Histological analysis

Paraffin-embedded LV were sectioned along the short axis into 5-µm-thick slices. After staining with hematoxylin-eosin (H.-E.), muscular degeneration and inflammation (mononuclear inflammatory infiltrate) in the infarct area were evaluated by one pathologist blinded to the genotypes. Ladewig staining was performed to assess the degree of fibrosis in the infarcted area.

9. Patients

An institutional review committee approved the study, and all patients gave written consent. Study patients were prospectively defined and subdivided into five groups: Group 1 - Chagas' disease without systolic ventricular dysfunction (LVEF > 50%); Group 2 - Chagas' disease with ventricular systolic dysfunction (LVEF <50%), in NYHA classes I-II; Group 3 - Chagas' disease with ventricular systolic dysfunction (LVEF <50%), in NYHA classes III-IV; Group 4 - DCM with ventricular systolic dysfunction (LVEF <50%), in NYHA classes I-II; and Group 5 - DCM with ventricular systolic dysfunction (LVEF <50%), in NYHA classes III-IV.

10. ANP and BNP measurements

ANP and BNP concentrations were determined in duplicate by highly sensitive and specific immunoradiometric assays (IRMA: Schering-Berlin, Germany).

11. Data and statistical analysis

All data were expressed as mean ± SEM. Differences between groups were determined using two-way ANOVA followed by post-hoc testing using Student's *t* test. Associations between the investigated peptides and clinical variables were analyzed by Mann-Whitney *U* test. The ability of the peptides ANP and BNP to discriminate between Chagas patients with high vs. low EF was described by receiver operator characteristics (ROC) curves drawn by plotting the sensitivity against the specificity for varying cut-off levels of ANP or BNP. Kaplan-Meier analysis was used to compare the survival of Chagas patients. A value of $P<0.05$ was considered statistically significant.

Results

1. Generation and basic characterization of CNP transgenic mice

Transgenic mice overexpressing rat CNP in cardiomyocytes were generated. The transgene integration in the chromosomal DNA was proven by Southern blot. Six independent lines were detected according to different patterns of integration into the genomic DNA. Since mRNA quantitative analysis showed that CNP mRNA expression was the highest in line 1 (TGM MHCCNP1) among the four investigated lines, this line was selected for the experiments.

To investigate the ontogenetic regulation of CNP transgene expression, cardiac CNP mRNA of transgenic mice was determined at different time points. CNP transgene overexpression was detected at all time points with no significant variation in aging. To exclude ectopic expression of the transgene, the CNP mRNA in atrium, ventricle, lung, kidney, liver, testis, forebrain, and hindbrain was detected in 3-month-old male TG mice and their WT littermates. As expected, high transgene CNP expression was detected in the atrium and ventricle, whereas none of the other investigated organs showed detectable transgene expression.

Peptide concentrations were measured in cardiac tissue, lung, kidney and plasma to confirm that CNP mRNA overexpression also led to more CNP generation. CNP levels were significantly increased in atria and ventricles of transgenic mice. No change was found in the other organs or plasma.

Due to its interaction with the NPRB receptor, which couples to guanylyl cyclase, CNP stimulates the generation of second messenger cGMP. While the cGMP level was significantly increased in ventricles of transgenic mice, it did not differ in plasma.

Furthermore, CNP overexpression in cardiomyocytes did not modify blood pressure and heart rate compared to their age-matched wild-type littermates.

2. Effect of CNP on I/R injury

To assess the impact of CNP overexpression on I/R injury, infarct size was measured after 1-hour regional myocardial ischemia and 23-hour reperfusion. AAR/LV was similar in both groups. No significant difference was observed in myocardial infarct size as defined as the percentage of IA/AAR compared to wild-type controls.

3. Analysis of cardiac remodeling and function after myocardial infarction

The LV weight/tibia length ratio did not differ for sham-operated WT and TG mice. All other measured parameters were comparable in sham-operated wild-type and transgenic mice. However, three weeks after MI, infarct-induced cardiac hypertrophy was prevented by CNP overexpression in cardiomyocytes. While LV mass was significantly increased in wild-type mice, no significant increase in LV weight was observed in mice with elevated CNP expression. The RV/tibia length ratio was also significantly increased in wild-type mice, but CNP overexpression in cardiomyocytes prevented MI-induced RV hypertrophy.

Cardiac function was significantly impaired in both MI groups except for HR and Tau. Whereas MAP was significantly decreased after MI in the wild-type group, it did not differ in transgenic mice. Moreover, indices of LV systolic and diastolic function, as dP/dt_{max} and dP/dt_{min}, fractional shortening, and LV_{EDD} indicated better performance in transgenic mice, although they did not reach statistical significance.

4. Quantification of parameters of cardiac failure

In the non-infarcted LV, mRNA levels of collagen type III were significantly elevated after MI in both groups without a significant inter-group difference. However, the increase in the transgenic group was less pronounced than in the wild-type mice. BNP mRNA, which is known to be upregulated in the ventricles under pathophysiological conditions like cardiac failure, was significantly elevated after MI in the wild-type group, while the increase in the transgenic group did not reach statistical significance.

5. Histological analysis

Normal cardiomyocytes were evident in both sham-operated lines. However, there was prominent muscular degeneration and a moderate mononuclear inflammatory infiltration in the infracted area of wild-type mice three weeks after MI compared to only mild degeneration of myofibrils and no mononuclear inflammatory infiltrate in the transgenic group.

No fibrosis was observed in sham-operated mice of either group. Marked fibrosis was observed in the infarcted area of both groups that underwent myocardial infarction surgery. However, confirming the findings of H.-E. staining, there were more abundant muscle fibers preserved in the TG group than in the wild-type controls.

6. ANP, BNP, and CNP in CD and DCM

In both CD and DCM patients, ANP and BNP plasma levels were significantly increased, and their increase was directly related to the NYHA classes, but ANP alteration was less

pronounced than the BNP. In contrast to ANP and BNP, circulating CNP concentration increased significantly only in patients with NYHA classes III and IV. Both ANP and BNP, but not CNP, plasma concentrations significantly predicted increased risk of death or the necessity for heart transplantation in Chagas patients.

Discussion

In the present study, transgenic mice were generated and characterized, demonstrating that cardiomyocyte-restricted CNP overexpression does not reduce infarct size produced by 1-hour global myocardial ischemia and 23-hour reperfusion, but prevents cardiac hypertrophy three weeks post myocardial infarction.

CNP mediates most of its physiological actions via interaction with its receptor NPRB. However, it was also recently identified as an endothelium-derived hyperpolarizing factor (EDHF) in rat mesenteric arteries involving activation of the clearance receptor NPRC (7). This CNP/NPRC signaling pathway has been implicated in the regulation of coronary blood flow and the reduction of I/R-induced infarct size in isolated perfused Langendorff rat hearts (4). In contrast to this described cardioprotective role of CNP against I/R, we did not observe a significant decrease in infarct size in present study. Species-specific differences, independent experimental protocols, and unequal origin of CNP elevation may account for this discrepancy. Firstly, Langendorff preparations from rat hearts were used in earlier studies, while we investigated transgenic mice *in vivo*. Secondly, mice were subjected to 1-hour ischemia and 23-hour reperfusion in our *in vivo* studies, but a protocol of 25 minutes of global ischemia and 120 minutes of reperfusion was used in previous *ex vivo* experiments. Thirdly, CNP originates from cardiomyocytes in our transgenic animals and thus acts in an autocrine way, but this peptide was supplied intravascularly in retrogradely perfused hearts, thereby exposing the endothelium to the highest concentrations. Thus, the discrepancy between Hobbs' *et al.* and our findings could be due to the necessity to stimulate NPRC on endothelial cells in order to mediate beneficial effects of CNP in an I/R model that did not occur in our transgenic mice.

Although we did not see beneficial effects of CNP on I/R injury in an *in vivo* model, cardiomyocyte-restricted overexpression of this peptide identified significantly improved cardiac remodeling three weeks after myocardial infarction. Both MI-induced LV and RV hypertrophy were prevented in our transgenic mice. The results provide clear *in vivo* evidence that CNP is a potent antihypertrophic agent after MI. This agrees in part with a previous report by Soeki and colleagues in rats (5). *In vivo* administration of CNP via osmotic mini-

pumps significantly improved cardiac function and led to less pronounced cardiac hypertrophy and fibrosis in rats two weeks post MI (5). In our present study, however, the improved cardiac remodeling in TG mice did not result in significantly improved cardiac function compared to their infarcted wild-type controls, although impairment of cardiac function compared to the sham group was less pronounced in transgenic mice than in the infarcted wild-type mice, probably due to inhibited cardiac hypertrophy. However, while they applied CNP by minipump, we exclusively elevated cardiac CNP concentrations, showing for the first time that the local cardiac CNP mediates the antihypertrophic effects.

The prevention of hypertrophy by CNP overexpression in our transgenic mice that underwent myocardial infarction is in agreement with data on antihypertrophic effects of its receptor NPRB, as recently reported by Langenickel *et al.* (8). Transgenic rats expressing a dominant-negative mutant of NPRB (NPR-BΔKC) develop progressive, blood pressure-independent cardiac hypertrophy (8). Histological assessment and echocardiography revealed cardiac hypertrophy in NPR-BΔKC transgenic rats, which was aggravated with age accompanied by increasing cardiac markers of heart failure. Interestingly, there was no evidence for increased interstitial or perivascular fibrosis in those rats, supporting our finding of non-significant differences in the grade of stimulated fibrosis post MI in CNP-transgenic mice compared to wild-type controls. Nevertheless, the combination of data from Langenickel *et al*. and our findings provides clear evidence that especially the CNP/NPRB axis is implicated in the regulation of cardiomyocyte growth but not in cardiac fibrosis.

Furthermore, the limitation of Langenickel *et al.*'s findings was the increased heart rate of NPR-BΔKC transgenic rats compared to controls caused by a possible mechanism of reduced CNP actions on the central nervous system facilitating baroreflexes by downregulation of functional NPRB. Therefore, they could not discriminate whether central or peripheral alterations are responsible for the cardiac hypertrophy observed in this animal model. Since our transgenic mice do not show altered systemic blood pressure and heart rate and specifically overexpress CNP in the heart, we can finally define the cardiac CNP/NPRB axis to be crucial in preventing cardiac hypertrophy.

Furthermore, the fact that the CNP/NPRB axis is responsible for the cardiac CNP effects is also supported by cell-based studies. CNP modulates the growth, proliferation, and hypertrophy of smooth muscle cells, cardiomyocytes and fibroblasts (9-11), and cGMP is implicated in all of these studies when CNP binds to its receptor NPRB.

Diagnostic and prognostic values of three natriuretic peptide plasma levels in CD and DCM patients were investigated. ANP and BNP have been established as markers for cardiac hypertrophy and cardiomyopathy, and BNP has become particularly important in the clinical diagnostic of cardiovascular diseases. As previously described for DCM, marked elevation of plasma ANP and BNP concentrations was observed, which was dependent on the NYHA class, the increase of plasma ANP levels being less pronounced than for BNP. For NYHA class III-IV patients, the peptide concentrations in Chagas disease were comparable to those measured in the DCM group and concordant with data reported in the literature. The fact that Chagas patients without cardiac dysfunction have already significantly elevated ANP and BNP levels suggests that they may be good markers to screen asymptomatic Chagas' disease without ventricular dysfunction for earlier diagnosis and treatment.

In conclusion, the present study indicates that the CNP/NPRB/cGMP signaling pathway plays an important role in the local regulation of cardiac hypertrophy under pathophysiological conditions. However, CNP failed to identify asymptomatic CD patients. In contrast, ANP and BNP are very potent markers to identify asymptomatic CD patients and to predict death or necessity for heart transplantation.

References

1. Stepan, H., Leitner, E., Bader, M. & Walther, T. (2000) *Regul Pept* **95,** 81-5.
2. Nakao, K., Ogawa, Y., Suga, S. & Imura, H. (1992) *J Hypertens* **10,** 1111-4.
3. D'Souza, S. P., Davis, M. & Baxter, G. F. (2004) *Pharmacol Ther* **101,** 113-29.
4. Hobbs, A., Foster, P., Prescott, C., Scotland, R. & Ahluwalia, A. (2004) *Circulation* **110,** 1231-5.
5. Soeki, T., Kishimoto, I., Okumura, H., Tokudome, T., Horio, T., Mori, K. & Kangawa, K. (2005) *J Am Coll Cardiol* **45,** 608-16.
6. Tarnavski, O., McMullen, J. R., Schinke, M., Nie, Q., Kong, S. & Izumo, S. (2004) *Physiol Genomics* **16,** 349-60.
7. Chauhan, S. D., Nilsson, H., Ahluwalia, A. & Hobbs, A. J. (2003) *Proc Natl Acad Sci U S A* **100,** 1426-31.
8. Langenickel, T. H., Buttgereit, J., Pagel-Langenickel, I., Lindner, M., Monti, J., Beuerlein, K., Al-Saadi, N., Plehm, R., Popova, E., Tank, J., Dietz, R., Willenbrock, R. & Bader, M. (2006) *Proc Natl Acad Sci U S A* **103,** 4735-40.
9. Furuya, M., Yoshida, M., Hayashi, Y., Ohnuma, N., Minamino, N., Kangawa, K. & Matsuo, H. (1991) *Biochem Biophys Res Commun* **177,** 927-31.
10. Rosenkranz, A. C., Woods, R. L., Dusting, G. J. & Ritchie, R. H. (2003) *Cardiovasc Res* **57,** 515-22.
11. Horio, T., Tokudome, T., Maki, T., Yoshihara, F., Suga, S., Nishikimi, T., Kojima, M., Kawano, Y. & Kangawa, K. (2003) *Endocrinology* **144,** 2279-84.

Curriculum Vitae

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Publication list

1. Heringer-Walther S, Moreira Mda C, Wessel N, **Wang Y**, Ventura TM, Schultheiss HP, Walther T. Does the C-type natriuretic peptide have prognostic value in chagas disease and other dilated cardiomyopathies? J Cardiovasc Pharmacol 2006;48:293-298.

2. **Wang Y**, de Waard MC, Sterner-Kock A, Stepan H, Schultheiss HP, Duncker DJ, Walther T. Cardiomyocyte-restricted over-expression of C-type natriuretic peptide prevents cardiac hypertrophy induced by myocardial infarction in mice. Eur J Heart Fail 2007;9:548-557.

3. Moreira Mda C, Heringer-Walther S, Wessel N, Ventura TM, **Wang Y**, Schultheiss HP, Walther T. Prognostic value of natriuretic peptides in Chagas' disease: a 3-year follow-up investigation. Cardiology 2007. (in press)

4. Walther T, Tschope C, Sterner-Kock A, Westermann D, Heringer-Walther S, Riad A, Nikolic A, **Wang Y**, Ebermann L, Siems WE, Bader M, Shakibaei M, Schultheiss HP, Dorner A. Accelerated mitochondrial adenosine diphosphate/adenosine triphosphate transport improves hypertension-induced heart disease. Circulation 2007;115:333-344.

5. Pankow K, **Wang Y**, Gembardt F, Krause E, Sun X, Krause G, Schultheiss HP, Siems WE, Walther T. A successive action of meprin A and neprilysin catabolizes B-type natriuretic peptide. Circ Res 2007;101:875-882.

Erklärung

„Ich, Yong, Wang, erkläre, dass ich die vorgelegte Dissertationsschrift mit dem Thema: ″Cardiovascular effects of C-type natriuretic peptide (CNP) overexpression in transgenic animals″ selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt, ohne die (unzulässige) Hilfe Dritter verfasst und auch in Teilen keine Kopien anderer Arbeiten dargestellt habe.“

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