Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 510 (2005) 209–215

www.elsevier.com/locate/ejphar

Effects of natriuretic peptides on ventricular myocyte contraction and role of cyclic GMP signaling

Qihang Zhang[a], Jacob Moalem[b], James Tse[c], Peter M. Scholz[b], Harvey R. Weiss[a,*]

[a]*Heart and Brain Circulation Laboratory, Department of Physiology and Biophysics, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, Piscataway, NJ 08854-5635, USA*

[b]*Heart and Brain Circulation Laboratory, Department of Surgery, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, Piscataway, NJ 08854-5635, USA*

[c]*Heart and Brain Circulation Laboratory, Department of Anesthesia, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, Piscataway, NJ 08854-5635, USA*

Received 7 October 2004; received in revised form 14 January 2005; accepted 14 January 2005

Abstract

Natriuretic peptides, including atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP) act through different receptors and at different potencies to affect cardiac myocyte function. We tested the hypothesis that these three peptides would differentially reduce cardiomyocyte function through their effects on the cyclic GMP signaling pathway. Rabbit ventricular myocytes were isolated and stimulated by electrical field stimulation. Cell function was measured using a video edge detector. ANP BNP or CNP at 10^{-9}, 10^{-8}, 10^{-7} M were added to the myocytes. Intracellular cyclic GMP was determined using a radioimmunoassay in the absence or presence of ANP, BNP or CNP. All natriuretic peptides decreased myocyte contractility in a similar concentration dependent manner. Myocyte percentage shortening was significantly decreased with all peptides at 10^{-7} M compared with baseline (ANP from 5.4±0.4 to 3.9±0.2%; BNP from 5.0±0.2 to 3.5±0.1%; CNP from 5.6±0.3 to 4.0±0.3%). Maximum rate of shortening and relaxation were also decreased similarly and significantly. Intracellular cyclic GMP was significantly increased in myocytes treated with ANP, BNP or CNP (Baseline 1.0±0.2, ANP 2.1±0.2, BNP 2.3±0.3, CNP 2.0±0.2 pmol/10^5 myocytes). Furthermore, inhibition of the cyclic GMP protein kinase with KT5823 caused a reversal in the functional effects of CNP. We concluded that all natriuretic peptides had similar negative effects on ventricular myocyte function and their effects were accompanied by increased cyclic GMP. Blockade the effect of CNP by a cyclic GMP protein kinase inhibitor demonstrated that effects were mediated through the cyclic GMP signaling pathway.

Keywords: Atrial natriuretic peptide; Brain natriuretic peptide; C-type natriuretic peptide; Cyclic GMP protein kinase; (Rabbit)

1. Introduction

Natriuretic peptides are a family of structurally related peptides with potent diuretic, natriuretic, and vasorelaxant activity. There are at least three members in natriuretic peptide family that have major effects on the cardiovascular system, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP) (Levin et al., 1998). All three peptides are expressed in heart. ANP and BNP are produced mainly the atrium and ventricle, respectively (D'Souza et al., 2004). The secretion of ANP and BNP are triggered by wall pressure and stretch as well as some hormones and neurotransmitters (De Bold et al., 2001). CNP is predominantly located in central nervous system, anterior pituitary, kidney and vascular endothelial cells (Chen and Burnett, 1998; Fowkes and McArdle, 2000).

ANP and BNP are ligands for the natriuretic receptor NPR1 (GC-A) and CNP binds to NPR2 (GC-B) (Tremblay et al., 2002). ANP has been reported to have a higher affinity than BNP for NPR1 (D'Souza et al., 2004). Binding

* Corresponding author. Tel.: +1 732 235 4626; fax: +1 732 235 5308.
E-mail address: hweiss@umdnj.edu (H.R. Weiss).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.01.031

of the natriuretic peptides to their receptors activate a particulate guanylyl cyclase that is part of the receptor, leading to an elevation in intracellular cyclic GMP and a subsequent cascade of events (Kuhn, 2004; Sharma, 2002). There is also a clearance receptor NPR3 that binds all three natriuretic peptides, but lacks a particulate guanylyl cyclase domain. NPR1 is the most abundant type in large blood vessels. ANP and BNP can regulate arterial blood pressure and volume homeostasis and also have local antihypertrophic actions in the heart through NPR1. CNP has a paracrine function in vascular regeneration and myocardial function (Chen and Burnett, 1998; Pierkes et al., 2002). These actions are related to the production of cyclic GMP (Kuhn, 2004; Sharma, 2002; Tremblay et al., 2002). In the heart, cyclic GMP usually produces negative functional and metabolic effects on cardiac myocytes (Gong et al., 1997; Shah and MacCarthy, 2000; Zhang et al., 2002). However, the effects of natriuretic peptides on cardiac myocyte function are more controversial with reports of increases, decreases and no change in myocyte function after administration of the various natriuretic peptides (D'Souza et al., 2004; Hirose et al., 1998; Nir et al., 2001). Thus, natriuretic peptides affect different receptors, have different potencies and may have differential effects on cardiac function.

We tested the hypothesis that the natriuretic peptides, acting through cyclic GMP, would have differential negative functional effects on cardiac myocytes. In this study, we compared ANP, BNP and CNP at same concentrations and examined their effects on isolated ventricular myocytes from the rabbit heart. We determined myocyte function and changes in cyclic GMP levels. We also investigated if the functional effects were associated with the cyclic GMP signaling pathway by the action of the natriuretic peptides on the cyclic GMP protein kinase. We found similar effects for all three natriuretic peptides on myocyte function and the level of cyclic GMP.

2. Materials and methods

This investigation was conducted in accordance with the guide for the Care of Laboratory Animals (DHHS Publication 85-23, revised 1996) and was approved by our Institutional Animal Care and Use Committee.

2.1. Ventricular myocyte dissociation

Ventricular myocytes were isolated from the hearts of New Zealand white rabbits (n=8, 1.5–2.5 kg) as previously described (Gong et al., 1997; Zhang et al., 2002). Briefly, the rabbits were anesthetized with sodium pentobarbital (35 mg/kg) followed by the administration of heparin (10 units/g body weight) intravenously using the circumflex ear vein. The heart was immediately removed after an overdose of pentobarbital (60 mg/kg) and retrograde perfused through the aorta with minimal essential medium (MEM, Sigma) supplemented with 10 mM taurine, 2 mM L-glutamic acid and 20 mM HEPES, pH 7.2. After 5 min of perfusion with MEM, the heart was perfused with MEM containing 0.1% type II collagenase (Worthington) for 16 min. All perfusion media were maintained at 37 °C and equilibrated with water saturated oxygen.

After collagenase perfusion, the heart was removed from the perfusion apparatus and the ventricle was cut into 8–10 pieces. The tissue suspension was further treated with MEM containing 0.1% collagenase and 0.5% bovine serum albumin (BSA), fraction V (Sigma) at 37 °C and gently swirled at 2 cycles/s for 5 min. A slurry containing isolated myocytes was decanted from the tissue suspension. The isolated cells were washed three times in MEM containing 0.5% BSA and centrifuged at low speed ($34\times g$) to completely remove the collagenase and subcellular debris. Incubation of the remaining tissue with collagenase was repeated at least two more times. Myocyte viability was assessed by maintenance of a rod-shaped morphology and was between 50% and 70%. Yields were typically 10–14×10^6 rod-shaped cells/heart.

2.2. Myocyte functional measurements

Individual ventricular myocytes were studied for function. Cells were suspended in 2 ml of 2 mM Ca^{2+} MEM solution containing 0.5% BSA maintained at 37 °C in a chamber that was fitted onto the stage of an inverted light microscope (Zeiss Axiovert 125, Carl Zeiss). Two platinum wires were placed on two parallel sides of the chamber and were used to pace the myocytes by electric field stimulation (1 Hz, 5 ms duration, voltage 10% above threshold, and polarity altered with each pulse). Unloaded shortening of selected cardiac myocytes was measured on-line using a video edge-detector system (Crystal Biotech, model VED-114, Patton Biomedical) and a camera which detected the change of position of both edges of the cell. Data were collected continuously. The output of the video edge detector was fed into a television monitor and a desktop computer and the data were subsequently analyzed. Cells used to determine the functional parameters were healthy and could react to different reagents throughout the course of the experiment. Cell contraction measurements were obtained on random cells in each preparation and each cell was required to complete its protocol. Untreated cells continued to contract at a constant level over the time course of the experiment.

2.3. Determination of cyclic GMP level

Myocytes were collected, and incubated under electric stimulation and gently swirled at 2 cycles/s at 37 °C for 5 min in absence or presence of 10^{-7} M ANP, 10^{-7} M BNP, or 10^{-7} M CNP. At the end the incubation, myocyte samples were centrifuged and pellets were frozen in liquid nitrogen immediately and stored in −80 °C.

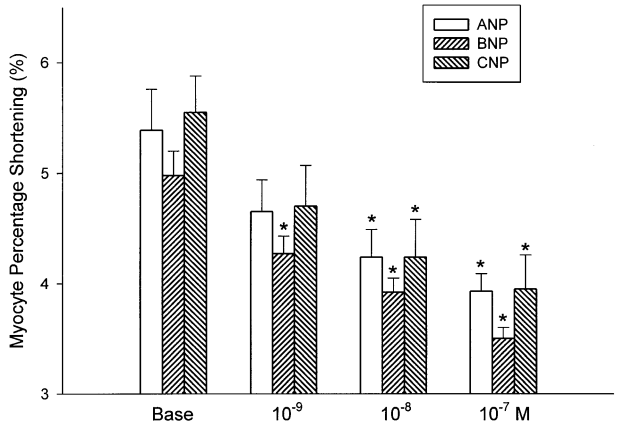

Fig. 1. Ventricular myocyte percentage shortening before and after cells were treated with ANP, BNP or CNP of 10^{-9}, 10^{-8}, 10^{-7} M. Note the similar dose dependent reduction in percent shortening with all the natriuretic peptides. Values represent mean±S.E.M. (N=8 rabbits, 3 cells/rabbit). *Significantly different from baseline.

To determine cyclic GMP levels, the myocytes were warmed to 0 °C and homogenized in ethanol using a Brinkmann Polytron (Westbury, NY) in an ice bath. The homogenate was centrifuged at 30,000×g for 15 min in a Sorvall RC-5B centrifuge (Dupont, Wilmington, DE). The supernatant was recovered. The pellet was resuspended in 1 ml of a 2 ethanol/1 water mixture and centrifuged as before. The combined supernatants were evaporated to dryness in a 60 °C bath under a stream of nitrogen gas. The final residue was dissolved in 1.5 ml assay buffer (0.05 mol/l sodium acetate, pH 5.8, containing sodium azide). Cyclic GMP levels were then determined using a radioimmunoassay (Amersham Pharmacia). This assay measures the competitive binding of [^{125}I]-labeled cyclic GMP to a cyclic GMP-specific antibody. After construction of a standard curve, cyclic GMP levels were determined and reported as picomoles/10^{-5} myocytes.

2.4. Experimental protocol

Ventricular myocytes were used in the following protocol for cell functional measurements. In all groups, myocytes in appropriate concentration were suspended in a chamber with 2 ml of MEM containing 2 mM Ca^{2+} and 0.5% BSA. After a 10-min stabilization period paced with electrical field stimulation, baseline contraction data for an individual myocyte were recorded. At 5-min intervals, reagents were added to the medium and allowed to diffuse to the cell while cell function was measured. ANP, BNP, or CNP (Calbiochem) was added sequentially at concentrations of 10^{-9}, 10^{-8}, 10^{-7} M. In separate experiments (N=5 rabbits), KT5823 (Sigma), a cyclic GMP protein kinase inhibitor, was given to myocytes treated with CNP of 10^{-9}, 10^{-8}, 10^{-7} M. A minimum of 10 consecutive contractions were used for each data point. For each protocol, at least 3 cells in each animal were repeatedly measured. Measurements obtained included resting cell length, absolute cell shortening, maximal rate of shortening, rate of cell relaxation, and calculated percentage of cell shortening.

2.5. Statistics

Results are expressed as means±S.E.M. A repeated measure analysis of variance (ANOVA) was used to compare variables measured under the experimental and control conditions. Duncan's post hoc procedure was used to compare differences between baseline and various treatments. In all cases, a value of P<0.05 was accepted as significant.

3. Results

3.1. ANP, BNP and CNP dose-dependently decreased ventricular myocyte contractility

We investigated the effects of the natriuretic peptides ANP, BNP, and CNP on cardiomyocytes function. When ANP 10^{-9}, 10^{-8}, 10^{-7} M was added to the ventricular myocytes, cell percentage shortening was significantly decreased in a dose-dependent manner from 5.4±0.4% at baseline to 3.9±0.2% in presence of 10^{-7} M ANP. BNP 10^{-7} M also decreased myocyte percentage shortening from 5.0±0.2% at baseline to 3.5±0.1% and CNP 10^{-7} M from 5.6±0.3% to 4.0±0.3% (Fig. 1).

The natriuretic peptides reduced the maximum rate of shortening of ventricular myocytes (Fig. 2). ANP significantly reduced the maximum rate of myocyte shortening at 10^{-8} M from 63.6±7.4 of base level to 50.7±3.7 μm/s. BNP 10^{-7} M also decreased maximum rate of shortening from 60.9±3.7 to 49.5±3.3 μm/s and CNP 10^{-7} M lowered maximum rate of shortening from 67.3±5.0 to 54.2±5.7 μm/s.

Similarly, these natriuretic peptides reduced the maximum rate of relaxation of ventricular myocytes (Fig. 3).

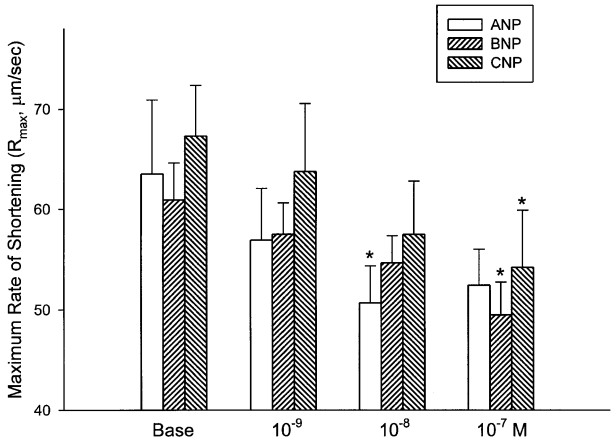

Fig. 2. Ventricular myocyte maximum rate of shortening before and after cells were treated with ANP, BNP or CNP of 10^{-9}, 10^{-8}, 10^{-7} M. Note the similar dose dependent reduction in maximum rate of shortening with all the natriuretic peptides. Values represent mean±S.E.M. (N=8 rabbits, 3 cells/rabbit). *Significantly different from baseline.

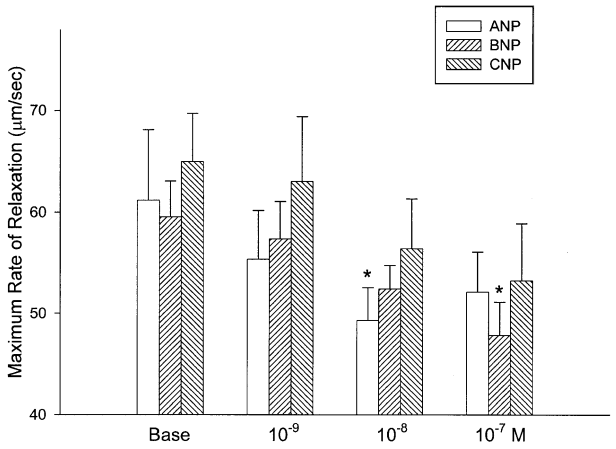

Fig. 3. Ventricular myocyte maximum rate of relaxation before and after cells were treated with ANP, BNP or CNP of 10^{-9}, 10^{-8}, 10^{-7} M. Note the similar dose dependent reduction in maximum rate of relaxation with all the natriuretic peptides. Values represent mean±S.E.M. (N=8 rabbits, 3 cells/rabbit). *Significantly different from baseline.

ANP at 10^{-7} M significantly reduced the myocyte maximum rate of relaxation from 61.2±6.9 of base level to 49.3±3.3 μm/s. BNP 10^{-7} M also decreased maximum rate of relaxation from 60.0±3.5 to 47.8±3.3 μm/s and CNP 10^{-7} M lowered maximum rate of relaxation from 65.0±3.7 to 53.2±5.7 μm/s.

3.2. ANP, BNP and CNP increased the intracellular cyclic GMP level of ventricular myocytes

To determine whether the effects of the natriuretic peptides were mediated through the cyclic GMP signaling pathway, cardiac myocytes were treated with ANP, BNP and CNP at 10^{-7} M and the intracellular cyclic GMP level was measured using radioimmunoassay. As shown in Fig. 4, all the natriuretic peptides significantly increased the ventricular myocyte intracellular cyclic GMP level. ANP 10^{-7} M raised the cyclic GMP level from 1.03±0.16 at baseline to

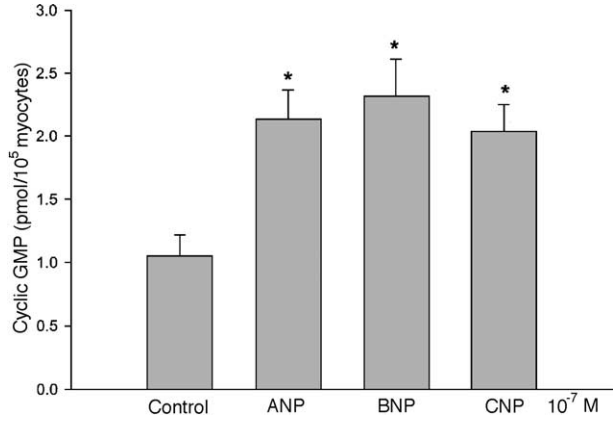

Fig. 4. Intracellular cyclic GMP levels of ventricular myocytes treated with vehicle or 10^{-7} M ANP, 10^{-7} BNP, or 10^{-7} M CNP. Note that all natriuretic peptides significantly and similarly increased intracellular cyclic GMP levels. Values represent mean±S.E.M. (N=6 rabbits, ~240,000±39,400 cells/rabbit). *Significantly different from control.

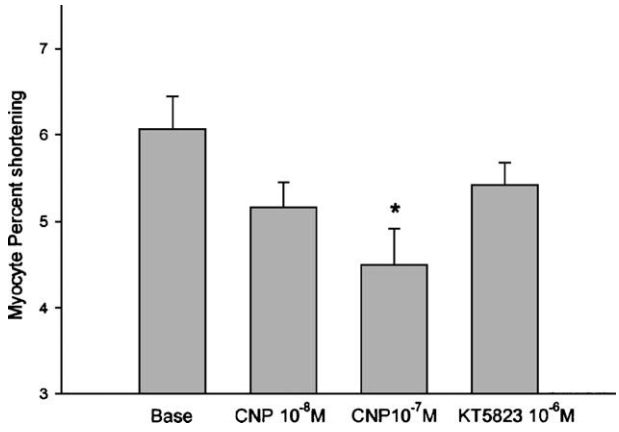

Fig. 5. Effect of CNP and the cyclic GMP protein kinase inhibitor KT5823 on myocyte percent shortening. Note that CNP significantly lowered percent shortening and KT5823 partially reversed this negative functional effect. Values represent mean±S.E.M. (N=5 rabbits, 3 cells/rabbit). *Significantly different from baseline.

2.13±0.22 pmol/10^{-5} myocytes. BNP 10^{-7} M and CNP 10^{-7} M also increased cyclic GMP to 2.31±0.29 and 2.03±0.21 pmol/10^{-5} myocytes correspondingly (Fig. 4).

3.3. Effects of CNP on myocyte contractility act via the cyclic GMP-dependent protein kinase

We further investigated whether the effects of the natriuretic peptides on myocyte contractility acted through cyclic GMP and the cyclic GMP-dependent protein kinase. CNP reduced percent shortening in a group of myocytes. Addition of KT5823, a specific inhibitor of the cyclic GMP protein kinase, reversed the CNP induced effect in reducing percent shortening of myocyte contraction (Fig. 5), demonstrating involvement of the cyclic GMP signaling pathway.

4. Discussion

The natriuretic peptides act on different receptors and at different potencies. The major finding of this study was that we found that all three natriuretic peptides, ANP, BNP and CNP, had similar effects on ventricular myocyte contractility, i.e. they all inhibited myocyte percentage shortening, maximum rate of shortening and relaxation to a similar degree. The effects of these natriuretic peptides were accompanied by a similar rise in myocyte intracellular cyclic GMP levels. Addition of a cyclic GMP protein kinase inhibitor reversed the effects of CNP on myocyte contractility further demonstrating involvement of the cyclic GMP/cyclic GMP protein kinase signaling pathway in the functional changes produced by natriuretic peptides in ventricular myocytes.

The use of isolated ventricular myocytes assured that cells were homogenous, experimental conditions were standardized and the results were related only to myocytes. The yields were high with 50–80% of rod shaped healthy

cells. The viability of the cells was confirmed by observing their shape and contractile responsiveness. The cells were able to contract for 30 min in the steady state (baseline level) in absence of any reagents. With regard to the functional measurements, we only chose cells that shortened at least 4%. Functional measurements were performed on three cells per group per heart. Using these isolated ventricular myocytes, we studied the relative contractile effects of the three natriuretic peptides.

Cyclic GMP, the intracellular signaling molecule common to nitric oxide and the natriuretic peptides, mediates an important signal transduction pathway in the cardiovascular system (Feil et al., 2003; Birschmann and Walter, 2004). Previous work had shown that cyclic GMP can reduce myocardial metabolism, inotropy and function (Gong et al., 1997; Shah and MacCarthy, 2000; Zhang et al., 2002). These negative functional effects of cyclic GMP in cardiac myocytes are mainly mediated through actions of the cyclic GMP-dependent protein kinase. The effects of cyclic GMP may also be mediated by a cyclic GMP protein kinase-independent interaction with other molecules in the cell, such as cyclic GMP-gated cation channels and certain cyclic AMP phosphodiesterases (Nascimento et al., 2001; Vila-Petroff et al., 1999; Wollert et al., 2002).

Guanylyl cyclases exist as both soluble and particulate, membrane-associated, enzymes that catalyze the conversion of GTP to cyclic GMP. The particulate guanylyl cyclase forms part of the receptors for the natriuretic peptides. These natriuretic receptors are membrane bound single chain transmembrane receptors that produce the second messenger cyclic GMP through either intra- or extracellular stimuli (Tremblay et al., 2002). The natriuretic receptors contain an intracellular catalytic guanylyl cyclase domain, an adjacent kinase-like domain and an extracellular (natriuretic peptide) ligand binding domain (Van den Akker, 2001). Binding of natriuretic peptides to their receptors induces a conformational change and activates these receptors leading to the production of cyclic GMP and the subsequent cascade of events.

The role of natriuretic peptides in the regulation of local cardiac contractility is controversial (D'Souza et al., 2004). Studies in genetic engineered mice demonstrate that the ANP/NPR1 system plays an autocrine/paracrine role in the heart, which inhibits cardiomyocyte growth and stimulates diastolic relaxation (Kishimoto et al., 2001; Holtwick et al., 2003). ANP has been reported to either have negative or no functional effects in heart or cardiac myocytes (D'Souza et al., 2004, Kuhn, 2004; Pierkes et al., 2002). Similar results have been reported for BNP (D'Souza et al., 2004). CNP has been shown to exert a biphasic, initially positive inotropic and lusitropic, then negative inotropic effect in isolated working mouse hearts (Wollert et al., 2003). Others have reported both positive and negative myocyte functional effects for CNP (Hirose et al., 1998; Nir et al., 2001). In our study, we found a similar dose dependent decrease in ventricular myocyte function with all three natriuretic peptides. To our knowledge, this is the first reported comparison of the cyclic GMP and functional effects of these three natriuretic peptides in ventricular myocytes. These differences may be related to species, dose or the various cardiac preparations used.

ANP and BNP both stimulate the NPR1 receptor, but ANP has been reported to be much more potent (D'Souza et al., 2004; Kuhn, 2004; Vanderheyden et al., 2004). However, this issue has not been studied in ventricular myocytes. We found that both ANP and BNP had approximately equal potency in increasing the level of cyclic GMP and reducing myocyte function. These peptides may play a complementary role in heart (Kuhn, 2004). CNP activates the NPR2 receptor (D'Souza et al., 2004; Vanderheyden et al., 2004). CNP also increased cyclic GMP and decreased myocyte function to a similar extent as ANP and BNP. This suggests that the NPR1 and NPR2 natriuretic peptide receptors have approximately equal potency in affecting signal transduction in rabbit ventricular myocytes.

The effects of the natriuretic peptides on myocardial function are related to production of cyclic GMP and activation of the cyclic GMP protein kinase (D'Souza et al., 2004; Kuhn, 2004). In the present study, we found that the negative functional effects of CNP were reversed by blocking the cyclic GMP protein kinase. A putative mechanism contributing to the contractile responses of the natriuretic peptides and cyclic GMP is the cyclic GMP protein kinase-dependent phosphorylation of phospholamban and subsequent activation of the sarcoplasmic reticulum Ca^{2+}-pump (Zhang et al., 2002). The cyclic GMP protein kinase has been shown to be a downstream target activated by the CNP/NPR2/cyclic GMP-signaling pathway in cardiac myocytes. Cyclic GMP protein kinase-stimulated phosphorylation of phospholamban and subsequent activation of the sarcoplasmic reticulum Ca^{2+} pump appears to mediate the responses of CNP in murine heart (Wollert et al., 2003). We showed that ANP, BNP and CNP at similar concentrations all decreased rabbit cardiac myocyte contractility and increased cyclic GMP levels. Our results support the idea that effects of the natriuretic peptides are mediated through the cyclic GMP protein kinase signaling mechanisms.

Stimulation of cyclic GMP synthesis by ANP/NPR1 or nitric oxide inhibits cardiomyocyte hypertrophy (Feil et al., 2003). The natriuretic peptides are upregulated in heart failure (Maisel et al., 2002). The plasma level of BNP has been correlated with heart failure. Therefore, it has been used as a diagnostic marker for heart failure (McCullough et al., 2003). It has been further developed as for clinical treatment of patients with congestive heart failure (Keating and Goa, 2003; Bhatia et al., 2003). Mice lacking ANP or the ANP receptor, NPR1, can develop pressure-independent cardiac hypertrophy (John et al., 1995). The hypertrophic response of cultured cardiac myocytes to adrenergic stimulation is suppressed by ANP, nitric oxide, or cyclic

GMP (Calderone et al., 1998). BNP has been used for treatment of acute decompensated congestive heart failure. Effects of BNP are associated with decreased preload and afterload and increased cardiac output (Iyengar et al., 2004). BNP and N-terminal BNP are also used to predict heart failure state and prognosis better than ANP or N-terminal ANP (Cowie et al., 1997; Maisel et al., 2002). Interestingly, our data demonstrate a similar effect for all three natriuretic peptides in terms of function and change in cyclic GMP. This suggests that other peptides or receptor agonists might also have a similar protective effect as BNP in acute decompensated cardiac failure.

In summary, we found that the natriuretic peptides, ANP, BNP and CNP, all decreased cell contractile function including percentage shortening, maximum rate of shortening and relaxation to a similar degree. Their effects on cardiac myocyte function were accompanied by doubling of the myocyte intracellular cyclic GMP level. These similar effects were observed despite the reported differences in potencies and receptor activated by these natriuretic peptides. Blockade of the cyclic GMP signaling pathway through the use of a cyclic GMP protein kinase inhibitor caused inhibition of effects of CNP. We conclude that the similar negative functional effects of these natriuretic peptides in ventricular myocytes are mediated through the cyclic GMP-cyclic GMP protein kinase signaling pathway.

Acknowledgements

This study was partially supported by NIH grant HL 40320.

References

Bhatia, V., Nayyar, P., Dhindsa, S., 2003. Brain natriuretic peptide in diagnosis and treatment of heart failure. J. Postgrad. Med. 49, 182–185.

Birschmann, I., Walter, U., 2004. Physiology and pathophysiology of vascular signaling controlled by guanosine 3′,5′-cyclic monophosphate-dependent protein kinase. Acta Biochim. Pol. 51, 397–404.

Calderone, A., Thaik, C.M., Takahashi, N., Chang, D.L., Colucci, W.S., 1998. Nitric oxide, atrial natriuretic peptide, and cyclic GMP inhibit the growth-promoting effects of norepinephrine in cardiac myocytes and fibroblasts. J. Clin. Invest. 101, 812–818.

Chen, H.H., Burnett Jr., J.C., 1998. C-type natriuretic peptide: the endothelial component of the natriuretic peptide system. J. Cardiovasc. Pharmacol. 32 (Suppl. 3), S22–S28.

Cowie, M.R., Struthers, A.D., Wood, D.A., Coats, A.J., Thompson, S.G., Poole-Wilson, P.A., Sutton, G.C., 1997. Value of natriuretic peptides in assessment of patients with possible new heart failure in primary care. Lancet 350, 1349–1353.

de Bold, A.J., Ma, K.K., Zhang, Y., de Bold, M.L., Bensimon, M., Khoshbaten, A., 2001. The physiological and pathophysiological modulation of the endocrine function of the heart. Can. J. Physiol. Pharm. 79, 705–714.

D'Souza, S.P., Davis, M., Baxter, G.F., 2004. Autocrine and paracrine actions of natriuretic peptides in the heart. Pharmacol. Ther. 101, 113–129.

Feil, R., Lohmann, S.M., de Jonge, H., Walter, U., Hofmann, F., 2003. Cyclic GMP-dependent protein kinases and the cardiovascular system: insights from genetically modified mice. Circ. Res. 93, 907–916.

Fowkes, R.C., McArdle, C.A., 2000. C-type natriuretic peptide: an important neuroendocrine regulator? Trends Endocrinol. Metab. 11, 333–338.

Gong, G.X., Weiss, H.R., Tse, J., Scholz, P.M., 1997. Cyclic GMP decreases cardiac myocyte oxygen consumption to a greater extent under conditions of increased metabolism. J. Cardiovasc. Pharmacol. 30, 537–543.

Hirose, M., Furukawa, Y., Kurogouchi, F., Nakajima, K., Miyashita, Y., Chiba, S., 1998. C-type natriuretic peptide increases myocardial contractility and sinus rate mediated by guanylyl cyclase-linked natriuretic peptide receptors in isolated, blood-perfused dog heart preparations. J. Pharmacol. Exp. Ther. 286, 70–76.

Holtwick, R., Van Eickels, M., Skryabin, B.V., Baba, H.A., Bubikat, A., Begrow, F., Schneider, M.D., Garbers, D.L., Kuhn, M., 2003. Pressure-independent cardiac hypertrophy in mice with cardiomyocyte-restricted inactivation of the atrial natriuretic peptide receptor guanylyl cyclase-A. J. Clin. Invest. 111, 1399–1407.

Kuhn, M., 2004. Molecular physiology of natriuretic peptide signaling. Basic Res. Cardiol. 99, 76–82.

Iyengar, S., Feldman, D.S., Trupp, R., Abraham, W.T., 2004. Nesiritide for the treatment of congestive heart failure. Expert Opin. Pharmacother. 5, 901–907.

John, S.W., Krege, J.H., Oliver, P.M., Hagaman, J.R., Hodgin, J.B., Pang, S.C., Flynn, T.G., Smithies, O., 1995. Genetic decreases in atrial natriuretic peptide and salt-sensitive hypertension. Science 267, 679–681.

Keating, G.M., Goa, K.L., 2003. Nesiritide: a review of its use in acute decompensated heart failure. Drugs 63, 47–70.

Kishimoto, I., Rossi, K., Garbers, D.L., 2001. A genetic model provides evidence that the receptor for atrial natriuretic peptide (guanylyl cyclase-A) inhibits cardiac ventricular myocyte hypertrophy. Proc. Natl. Acad. Sci. U. S. A. 98, 2703–2706.

Levin, E.R., Gardner, D.G., Samson, W.K., 1998. Natriuretic peptides. N. Engl. J. Med. 339, 321–328.

Maisel, A.S., Krishnaswamy, P., Nowak, R.M., McCord, J., Hollander, J.E., Duc, P., Omland, T., Storrow, A.B., Abraham, W.T., Wu, A.H., Clopton, P., Steg, P.G., Westheim, A., Knudsen, C.W., Perez, A., Kazanegra, R., Herrmann, H.C., McCullough, P.A., 2002. Breathing not properly multinational study investigators. Rapid measurement of B-type natriuretic peptide in the emergency diagnosis of heart failure. N. Engl. J. Med. 347, 161–167.

McCullough, P.A., Omland, T., Maisel, A.S., 2003. B-type natriuretic peptides: a diagnostic breakthrough for clinicians. Rev. Cardiovasc. Med. 4, 72–80.

Nascimento, J.H., Salle, L., Hoebeke, J., Argibay, J., Peineau, N., 2001. cGMP-mediated inhibition of cardiac L-type Ca^{2+} current by a monoclonal antibody against the M_2 ACh receptor. Am. J. Physiol. 281, C1251–C1258.

Nir, A., Zhang, D.F., Fixler, R., Burnett Jr., J.C., Eilam, Y., Hasin, Y., 2001. C-type natriuretic peptide has a negative inotropic effect on cardiac myocytes. Eur. J. Pharmacol. 412, 195–201.

Pierkes, M., Gambaryan, S., Boknik, P., Lohmann, S.M., Schmitz, W., Potthast, R., Holtwick, R., Kuhn, M., 2002. Increased effects of C-type natriuretic peptide on cardiac ventricular contractility and relaxation in guanylyl cyclase A-deficient mice. Cardiovasc. Res. 53, 852–861.

Shah, A.M., MacCarthy, P.A., 2000. Paracrine and autocrine effects of nitric oxide on myocardial function. Pharmacol. Ther. 86, 49–86.

Sharma, R.K., 2002. Evolution of the membrane guanylate cyclase transduction system. Mol. Cell. Biochem. 230, 3–30.

Tremblay, J., Desjardins, R., Hum, D., Gutkowska, J., Hamet, P., 2002. Biochemistry and physiology of the natriuretic peptide receptor guanylyl cyclases. Mol. Cell. Biochem. 230, 31–47.

van den Akker, F., 2001. Structural insights into the ligand binding domains of membrane bound guanylyl cyclases and natriuretic peptide receptors. J. Mol. Biol. 311, 923–937.

Vanderheyden, M., Bartunek, J., Goethals, M., 2004. Brain and other natriuretic peptides: molecular aspects. Eur. J. Heart Fail. 6, 261–268.

Vila-Petroff, M.G., Younes, A., Egan, J., Lakatta, E.G., Sollott, S.J., 1999. Activation of distinct cAMP-dependent and cGMP-dependent pathways by nitric oxide in cardiac myocytes. Circ. Res. 84, 1020–1031.

Wollert, K.C., Fiedler, B., Gambaryan, S., Smolenski, A., Heineke, J., Butt, E., Trautwein, C., Lohmann, S.M., Drexler, H., 2002. Gene transfer of cGMP-dependent protein kinase I enhances the antihypertrophic effects of nitric oxide in cardiomyocytes. Hypertension 39, 87–92.

Wollert, K.C., Yurukova, S., Kilic, A., Begrow, F., Fiedler, B., Gambaryan, S., Walter, U., Lohmann, S.M., Kuhn, M., 2003. Increased effects of C-type natriuretic peptide on contractility and calcium regulation in murine hearts overexpressing cyclic GMP-dependent protein kinase I. Br. J. Pharmacol. 140, 1227–1236.

Zhang, Q., Yan, L., Weiss, H.R., Scholz, P.M., 2002. Cyclic GMP-induced reduction in cardiac myocyte function is partially mediated by activation of the sarcoplasmic reticulum Ca^{2+}-ATPase. Pharmacology 64, 106–112.