Effects of Brain Natriuretic Peptide on Contraction and Intracellular Ca^{2+} in Ventricular Myocytes from the Streptozotocin-Induced Diabetic Rat

FRANK C. HOWARTH, NOURA AL SHAMSI, MARYAM AL QAYDI, MARIAM AL MAZROUEI, ANWAR QURESHI, S.I. CHANDRANATH, ELSADIG KAZZAM, AND ABDU ADEM

Faculty of Medicine and Health Sciences, United Arab Emirates University, Al Ain, UAE

ABSTRACT: The streptozotocin (STZ)-treated rat is a widely studied experimental model of diabetes mellitus (DM). Its pathophysiology includes hypoinsulinemia, hyperglycemia, cardiac hypertrophy, and a cardiomyopathy that is characterized by the presence of diastolic and/or systolic contractile dysfunction. As part of their endocrine function cardiomyocytes in the heart produce and secrete a family of related peptide hormones called the natriuretic peptides that include A-type natriuretic peptide (ANP) and B-type natriuretic peptide (BNP). ANP and BNP levels are variously augmented in patients with hypertension, cardiac overload, in the ventricles of failing or hypertrophied heart, in cardiac heart failure, in acute myocardial infarction (MI), and in some circumstances in DM. In this article, the effects of BNP on ventricular myocyte contraction and Ca^{2+} transport in STZ-induced diabetic rats have been investigated. BNP concentration was significantly increased in blood plasma and in atrial muscle in STZ-induced diabetic rats compared to age-matched controls. BNP was 11.9 $\pm$ 0.9 ng/mL in plasma from diabetic rats compared to 6.7 $\pm$ 1.6 ng/mL in controls and 15.8 $\pm$ 2.0 ng/mg protein in diabetic atrial muscle compared to 8.5 $\pm$ 1.0 ng/mg protein in controls. The heart weight to body weight ratio, an indicator of hypertrophy, was significantly increased in diabetic rat heart (4.3 $\pm$ 0.1 mg/g) compared to controls (3.7 $\pm$ 0.04 mg/g). The amplitude of shortening was not significantly altered in diabetic myocytes (10.3 $\pm$ 0.4%) compared to controls (10.9 $\pm$ 0.4%). BNP reduced the amplitude of shortening to a greater extent in diabetic myocytes (8.1 $\pm$ 0.6%) compared to controls (10.1 $\pm$ 0.4%). The time to peak (TPK) shortening was significantly prolonged in diabetic myocytes (254 $\pm$ 8 ms) compared to controls (212 $\pm$ 5 ms) and was not additionally altered by BNP. The time to half relaxation of shortening was

Address for correspondence: Dr. Frank C. Howarth, Department of Physiology, Faculty of Medicine and Health Sciences, United Arab Emirates University, P.O. Box 17666, Al Ain, UAE. Voice: 00-9713-7137536; fax: 00-9713-7671966.
e-mail: chris.howarth@uaeu.ac.ae

Ann. N.Y. Acad. Sci. 1084: 155–165 (2006).
doi: 10.1196/annals.1372.007

also significantly prolonged in diabetic myocytes (131 ± 8 ms) compared to controls (111 ± 5 ms). BNP (10^{-8} to 10^{-6} M) normalized the time to half relaxation of shortening in diabetic myocytes to that of controls. Time to peak (TPK) shortening of Ca^{2+} was not different between diabetic and control rats. However, BNP (10^{-7} M) increases TPK of Ca^{2+} significantly. The amplitude of the Ca^{2+} transient was significantly increased in diabetic myocytes (0.42 ± 0.02 Ratio units [RU]) compared to controls (0.36 ± 0.02 RU) and was not additionally altered by BNP. BNP may have a protective role in STZ-induced diabetic rat heart.

KEYWORDS: **diabetes mellitus; heart; BNP; ventricular myocytes; contraction; calcium**

INTRODUCTION

As in many other parts of the world diabetes is a major health concern in the United Arab Emirates. Epidemiological data suggest that the prevalence of diabetes among UAE nationals has increased dramatically in recent years. Data from the Emirates National Diabetic Survey 2000 suggest that 24% of UAE citizens have diabetes. Cardiovascular complications are the most common causes of morbidity and mortality in diabetic patients.[1] Both type 1 and type 2 diabetic patients have an increased risk of ischemic heart disease (IHD) and congestive heart failure (CHF). The streptozotocin (STZ)-treated rat is a widely studied experimental model of diabetes mellitus (DM). Its pathophysiology includes hypoinsulinemia, hyperglycemia, cardiac hypertrophy, and a cardiomyopathy that is characterized by the presence of diastolic and/or systolic contractile dysfunction. Defective Ca^{2+} signaling pathways including altered L-type Ca^{2+} current, Na^{+}/Ca^{2+} exchange, Ca^{2+}-ATPase, sarcoplasmic reticulum Ca^{2+} uptake/release mechanisms, and altered myofilament Ca^{2+} sensitivity partly underlie contractile dysfunction in the STZ-induced diabetic rat.[2–5] The heart in addition to being a very efficient pump is also an important endocrine organ. As part of its endocrine function, cardiomyocytes in the heart produce and secrete a family of related peptide hormones called the natriuretic peptides. This family includes A-type natriuretic peptide (ANP), B-type natriuretic peptide (BNP), C-type natriuretic peptide (CNP), and urodilatin. In contrast to ANP, which originates mainly from atrial tissue, BNP is expressed in both the atria and the ventricles, but is mainly released from the ventricles.[6] Ventricular proBNP production is strongly upregulated in cardiac failure and locally in the area surrounding a myocardial infarction (MI). ANP and BNP have potent diuretic, natriuretic, and vasodilatory activities via a guanylyl cyclase (GC)-coupled natriuretic peptide receptor subtype, GC-A.[7] Pathophysiologically, ANP and BNP levels are variously augmented in patients with hypertension, cardiac overload, in the ventricles of failing or hypertrophied heart, in CHF, in acute MI, and in DM under some circumstances.[6] Natriuretic peptides have emerged as important candidates for development of

diagnostic tools and therapeutic agents in cardiovascular disease. The hypothesis is that BNP serves to modulate muscle contraction and has a protective role in diabetic cardiomyopathy. In this article, the levels of BNP in blood plasma and in atrial and ventricular muscle from STZ-induced diabetic rats and age-matched controls have been measured. The effects of BNP on ventricular myocyte shortening and Ca^{2+} transport in STZ-induced diabetic rat have been investigated.

METHODS

Induction of Diabetes

Diabetes was induced by a single intraperitoneal injection of STZ (60 mg/kg; Sigma, Taufkirchen, Germany) administered to young male Wistar rats (200–250 g; bred inhouse). The STZ was dissolved in a citrate buffer solution (0.1 mol/L citric acid, 0.1 mol/L sodium citrate; pH 4.5). Age-matched controls received an equivalent volume of the citrate buffer solution alone. Both groups of animals were maintained on the same diet and water *ad libitum* until they were used 8–12 weeks later. Principles of laboratory animal care were followed throughout. Approval for this project was obtained from the Faculty of Medicine and Health Sciences Ethics Committee.

Measurement of BNP in Blood Plasma and in Atria and Ventricles

The measurement of BNP from serum or plasma and tissue homogenates was performed as described by the manufacturing company (Peninsula Laboratories, San Carlos, CA). Briefly, a curve is constructed first using various dilutions of the standard concentrate provided in the kit. Twenty-five microliters of primary antisera were dispensed into each well of the immunoplate and incubated for 1 h at room temperature. Fifty microliters of the unknown samples were dispensed into the designated wells and incubated for another 2 h at room temperature. The rehydrated biotinylated peptide (25 μL) was added to each well and gently agitated. The plate was covered with an acetate plate sealer (APS) and incubated overnight at 2–8°C. The APS was removed and the contents were discarded and washed five times with assay buffer and blot dry. One hundred microliters of the diluted streptavidin-conjugated horse radish peroxidase (SA-HRP) solution was dispensed into each well and the plate was resealed with APS. After incubating for 60 min the wells were washed five times and blot dry. One hundred microliters of the tetramethyl benzidine dihydrochloride (TMB) solution was added into each well and incubated for 0.5–1 h at room temperature until the solution turned blue. The reaction was stopped by adding 100 μL of 2N HCl into each well. When the color became blue the immunoplate was loaded onto a microtiter plate reader and the absorbance was read at 450 nm.

A standard plot of optical density versus concentration was drawn. The concentration of peptide in the unknown sample was determined by locating the sample's OD on the y axis of the standard curve.

Ventricular Myocytes Isolation

Single ventricular myocytes were isolated according to the previously described technique.[3] In brief, rats were killed humanely by cervical dislocation following stunning and their hearts were removed quickly and mounted on a Langendorff apparatus. Hearts were perfused retrogradely at a constant flow of 8 mL.g heart^{-1}.min^{-1} with a HEPES-based isolation solution containing 0.75 mM Ca^{2+}. Perfusion flow rate was adjusted to allow for differences in heart weight between STZ-treated and control animals. When the coronary circulation had cleared of blood, perfusion was continued for 4 min with a Ca^{2+}-free isolation solution containing 0.1 mM EGTA, and then for 6 min with solution containing 0.05 mM Ca^{2+}, 0.75 mg/mL collagenase (type 2; Worthington, Lakewood NJ), and 0.075 mg/mL protease (type 14; Sigma). Ventricles were then excised from the heart, minced and gently shaken in collagenase-containing isolation solution supplemented with 1% BSA. Cells were filtered from this solution at 4-min intervals and resuspended in 0.75 mM Ca^{2+}-containing isolation solution. Cell viability, defined as the percentage of rod-shaped myocytes in the cell suspension was recorded within 1 h of completing the cell isolation.

Ventricular Myocyte Experiments

Freshly isolated myocytes suspended in 0.75 mM Ca^{2+}containing isolation solution were divided into two aliquots, centrifuged at low speed and the cell pellets resuspended either in normal tyrode or normal tyrode containing BNP at various concentrations (Sigma, A-8208). Cells were incubated in BNP for 1 h before the commencement of experiments. For electrophysiological studies myocytes were allowed to settle on the glass bottom of a perspex chamber mounted on the stage of an inverted microscope (Axiovert 35, Zeiss, Germany). Myocytes were superfused (3–5 mL/min) with a HEPES-based normal tyrode solution containing 1 mM Ca^{2+} or normal tyrode containing BNP. Experiments were performed in electrically stimulated myocytes (1 Hz) at room temperature (23–25°C). Unloaded myocytes shortening was followed using a video edge detection system (VED-114, Crystal Biotech, Northborough, MA). The degree of shortening (expressed as a percentage of resting cell length [RCL]), the time to peak (TPK) shortening, and the time from peak to half (THALF) relaxation of shortening were recorded. Intracellular Ca^{2+} was measured in cells loaded with the fluorescent indicator fura-2 AM (F-1221, Molecular Probes, Eugene, OR) as described previously.[3] To measure intracellular Ca^{2+} concentration,

myocytes were alternately illuminated by 340 nm and 380 nm light using a monochromator (Cairn Research, Faversham, Kent, UK) that changed the excitation light every 2 ms. The resultant fluorescent emission at 510 nm was recorded by a photomultiplier tube and the ratio of the emitted fluorescence at the two excitation wavelengths (340/380 ratio) was calculated to provide an index of intracellular Ca^{2+} concentration.

Solutions

The cell isolation solution contained (in mM) 130.0 NaCl, 5.4 KCl, 1.4 $MgCl_2$, 0.4 NaH_2PO_4, 5 HEPES, 10 glucose, 20 taurine, and 10 creatine set to pH 7.3 with NaOH. The normal tyrode solution contained (in mM) NaCl 140, KCl 5, $MgCl_2$ 1, glucose 10, HEPES 5, and $CaCl_2$ 1 set to pH 7.4 with NaOH.

Statistics

Results were expressed as the mean ± SEM of "n" observations. "n" refers either to the number of rats or number of cells. Statistical comparisons were performed using either independent samples *t*-test or analysis of variance (ANOVA) followed by Bonferroni-corrected *t*-tests for multiple comparisons, as appropriate. $P < 0.05$ was considered to indicate a significant difference. Statistical analysis was carried out using SPSS (Chicago, IL).

RESULTS

General Characteristics of STZ-Induced Diabetic Rat

The general characteristics of STZ-treated rats compared with their age-matched controls are shown in TABLE 1. Diabetes was confirmed in STZ- treated rats by a significant, fivefold, elevation of blood glucose. STZ-treated rats characteristically had significantly lower body weights and heart weights compared with controls.

TABLE 1. General characteristics of STZ-induced diabetic rat

	Control	STZ
Body weight (g)	316.0 ± 16.4 (6)	266.2 ± 9.9 (5)*
Heart weight (g)	1.17 ± 0.05 (6)	1.14 ± 0.04 (5)
Blood glucose (mg/dL)	73.0 ± 5.9 (6)	327.2 ± 23.3 (5)**
Heart weight/body weight (mg/g)	3.70 ± 0.04 (6)	4.29 ± 0.11 (5)**

Data are mean ± SE. Numbers in parenthesis are numbers of animals. Statistical comparisons were performed with independent samples *t*-test. $^*P < 0.05$, $^{**}P < 0.01$.

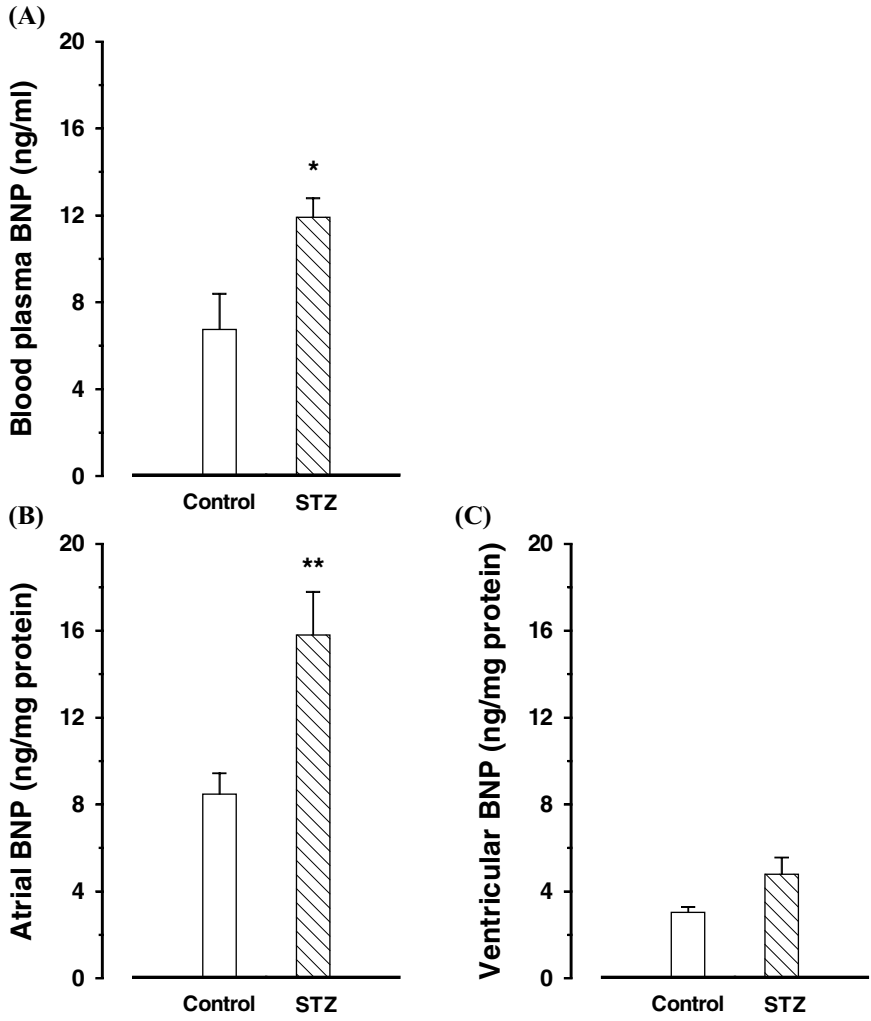

FIGURE 1. Effects of STZ-induced diabetes on BNP concentration in blood plasma (**A**), atrial muscle (**B**), and ventricular muscle (**C**). Data are mean ± SEM, $n = 4$. Statistical comparisons made with independent samples t-test ($^{*}P < 0.05$, $^{**}P < 0.01$).

Levels of BNP in Blood Plasma and in Ventricular and Atrial Muscle

The concentrations of BNP in blood plasma (panel A), and atrial (panel B), and ventricle (panel C) muscle are shown in FIGURE 1. The concentration of BNP was significantly ($P < 0.05$) increased in blood plasma and in atrial muscle and increased to a smaller extent in ventricular muscle from STZ-induced diabetic rat compared to age-matched controls. The concentration of

BNP in blood plasma from STZ-treated rats was 11.91 ± 0.89 ng/mL ($n = 4$) compared to 6.75 ± 1.64 ng/mL ($n = 4$) in controls. The level of BNP in atrial muscle from STZ-treated rats was 15.80 ± 2.00 ng/mg protein ($n = 4$ hearts) compared to 8.47 ± 0.97 ng/mg protein ($n = 4$ hearts) in controls. The level of BNP in ventricular muscle from STZ-treated rat was 4.78 ± 0.80 ng/mg protein ($n = 4$ hearts) compared to 3.03 ± 0.26 ng/mg protein ($n = 4$ hearts) in controls.

Effects of BNP on Ventricular Myocyte Shortening

A typical fast-time base recording of shortening in an electrically stimulated (1 Hz) myocyte superfused with normal tyrode containing 1 mM Ca^{2+} from a STZ-treated rat and a control rat is shown in FIGURE 2 A. The mean RCL of myocytes from STZ-treated (115.9 ± 2.9 μm, $n = 35$) was not significantly ($P > 0.05$) different from that of controls (121.9 ± 3.8 μm, $n = 33$). Exposure to 10^{-7} M BNP for at least 1 h did not significantly alter RCL. The time course of contraction was characteristically and significantly ($P < 0.05$) prolonged in diabetic myocytes compared to controls (FIG. 2 B, C). The mean TPK shortening was 254 ± 8 ms ($n = 35$) in diabetic myocytes compared to 212 ± 5 ms ($n = 33$) in controls (FIG. 2 B). Exposure of cells with 10^{-7} M BNP had no significant additional effect on TPK shortening in myocytes from either STZ-treated or control rats. The mean THALF relaxation was 131 ± 8 ms, $n = 35$ in diabetic myocytes compared to 111 ± 5 ms ($n = 33$) in controls (FIG. 2 C). Interestingly, when cells were exposed to BNP (10^{-8} to 10^{-7} M), the THALF relaxation in diabetic myocytes was normalized so that the difference in THALF relaxation between cells from diabetic myocytes and controls was no longer significant. The amplitude of shortening, expressed as a percentage of RCL, was slightly smaller in myocytes from STZ-treated (10.3 ± 0.4%, $n = 35$) rats compared to controls (10.9 ± 0.4%, $n = 33$) though the difference did not reach significance. Interestingly, exposure to BNP (10^{-7} and 10^{-6} M) produced a reduction in the amplitude of shortening (FIG. 2 D) that was significantly greater in diabetic myocytes compared to controls. At a concentration of 10^{-7} M BNP amplitudes of shortening were 8.1 ± 0.6% ($n = 22$) in diabetic myocytes compared to 10.1 ± 0.4% ($n = 38$) in controls.

Effects of BNP on Ventricular Myocyte Intracellular Ca^{2+}

Typical fast-time base recordings of Ca^{2+} transients in control and diabetic myocytes superfused with normal tyrode are shown in FIGURE 3 A. The mean resting fura-2 ratio in myocytes was not significantly altered by STZ treatment. The mean resting fura-2 ratio in diabetic myocytes was 2.43 ± 0.03 Ratio units (RU) ($n = 31$) compared to 2.34 ± 0.04 RU ($n = 28$) in controls. BNP (10^{-7} M) caused a significant reduction in mean

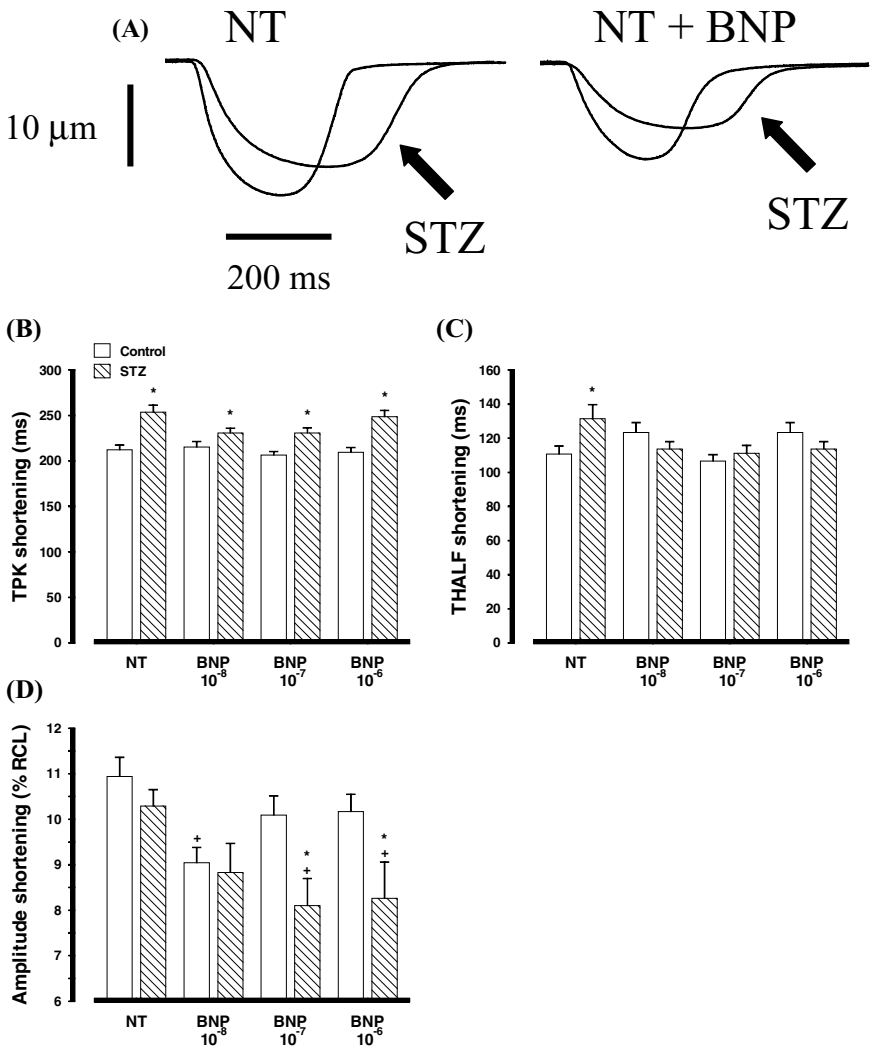

FIGURE 2. Effects of BNP on ventricular myocyte shortening. Typical records showing effects of STZ treatment and BNP on myocyte shortening (**A**). Graphs of TPK shortening (**B**), THALF relaxation of shortening (**C**), and amplitude of shortening (**D**). Data are mean ± SEM, $n = 20$–38. Means statistically compared using either independent samples t-test ($^{*}P < 0.05$) or ANOVA followed by Bonferroni-corrected t-tests ($^{+}P < 0.05$).

resting fura-2 ratio in myocytes from control rats (2.17 ± 0.03 RU, $n = 35$) though not in diabetic myocytes (2.41 ± 0.04 RU, $n = 30$). The time course of the Ca^{2+} transient was slightly prolonged in myocytes from STZ-treated rats compared to controls though the difference did not reach significance.

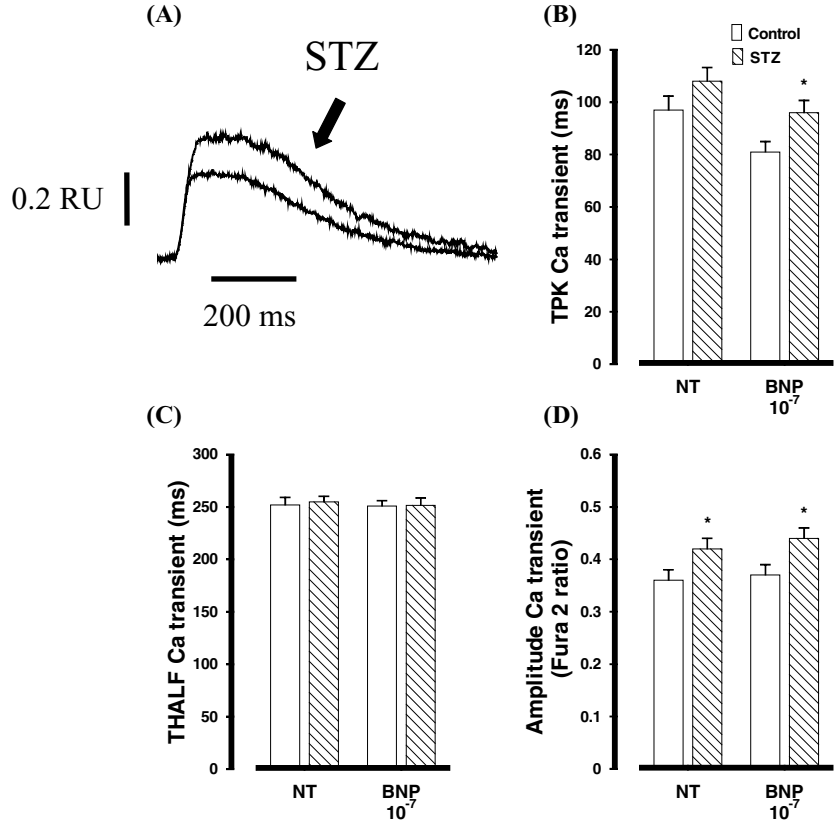

FIGURE 3. Effects of BNP on ventricular myocytes intracellular Ca^{2+}. Typical records showing effects of STZ treatment on the Ca^{2+} transient (**A**). Graphs of TPK Ca^{2+} transient (**B**), THALF relaxation of the Ca^{2+} transient (**C**), and amplitude of the Ca^{2+} transient (**D**). Data are mean ± SEM, $n = 28$–35. Mean statistically compared using Independent samples *t*-test ($^*P < 0.05$).

The TPK Ca^{2+} transient was 108 ± 5 ms ($n = 31$) in diabetic myocytes compared to 97 ± 5 ms ($n = 30$) in controls (FIG. 3 B). Interestingly, 10^{-7} M BNP reduced the TPK Ca^{2+} transient in both diabetic and in control myocytes. However, the reduction in TPK Ca^{2+} transient only reached significance in diabetic myocytes. The THALF relaxation of the Ca^{2+} transient was not significantly altered by either STZ treatment or by exposure to BNP. The amplitude of the Ca^{2+} transient was significantly increased (FIG. 3 D) in diabetic myocytes (0.42 ± 0.02 RU, $n = 31$) compared to controls (0.36 ± 0.02 RU, $n = 28$). Exposure to 10^{-7} M BNP had no additional effects in myocytes from either diabetic or control rats.

DISCUSSION

The study investigated the effects of STZ-induced diabetes on the distribution of BNP in plasma, and atrial and ventricular muscle. It also investigated the effects of BNP on ventricular myocyte shortening and intracellular Ca^{2+}. The major findings were that: (*a*) BNP concentrations were significantly elevated in blood plasma and atrial muscle and elevated to a smaller extent in ventricular muscle from STZ-treated rats compared to age-matched controls; (*b*) the time course, including TPK shortening and THALF relaxation of shortening was characteristically prolonged in diabetic myocytes compared to controls; (*c*) the amplitude of shortening was reduced by 10^{-7} M BNP and the effects were greater in diabetic myocytes compared to controls; and (*d*) the TPK Ca^{2+} transient was prolonged to a small extent in diabetic myocytes compared to controls and was reduced to a small extent in both diabetic and control myocytes in the presence of 10^{-7} M BNP. The reduction in TPK Ca^{2+} transient only reached significance in diabetic myocytes.

BNP concentration in plasma and atrial muscle increased significantly and to a smaller extent in ventricular muscle from diabetic rat heart compared to age-matched controls. Previous studies have demonstrated increased cardiac BNP expression in STZ-induced diabetic rat and increased expression of BNP mRNA in atrial myocardium but not in ventricular myocardium in STZ-induced diabetic pig.[8,9]

The heart weight to body weight ratio was increased in diabetic rats compared to age-matched controls. An increase in heart weight to body weight ratio is indicative of cardiac hypertrophy. Cardiac hypertrophy in STZ-induced diabetic rat has also been reported in some other studies.[10–12]

Plasma and atrial BNP were increased in diabetic rats compared to controls. This finding is consistent with previous data that have variously reported increases in ANP, ANP mRNA, and BNP expression.[10,12,13]

Contractile function is compromised in the STZ-induced diabetic rat heart. *In vivo* studies have demonstrated reduced heart rate, reduced left ventricular peak ejection rate and peak-filling rate, longer left ventricular ejection time, and isovolumic relaxation time.[2,14] Reductions in heart rate, left ventricular rate of development of systolic pressure, and rate of decline of the pressure are lower and TPK pressure and THALF relaxation from peak pressure longer in isolated perfused diabetic rat heart compared to controls.[2] Prolonged time course of contraction including prolonged TPK and THALF relaxation of contraction, seen in this study, and reduced amplitude of contraction are also frequently reported findings in ventricular myocytes from diabetic rat heart compared to controls.[2,3] Defects in Ca^{2+} transport including L-type Ca^{2+} current, sarcoplasmic reticulum Ca^{2+} release and uptake mechanisms, and Na^{+}/Ca^{2+} exchange partly underlie defects in contraction.[2,3] Interestingly, superfusion of myocytes with 10^{-7} M BNP reduced the amplitude of contraction in myocytes

from control and from diabetic rats though the effects of BNP were greater in myocytes from diabetic rat heart.

In conclusion, the increase in BNP seen in plasma and in atrial muscle and the increased effects of BNP on amplitude of shortening and TPK Ca^{2+} transient in myocytes from diabetic heart may suggest a protective role for BNP in STZ-induced diabetic rat heart.

REFERENCES

1. AMOS, A.F., D.J. MCCARTY & P. ZIMMET. 1997. The rising global burden of diabetes and its complications: estimates and projections to the year 2010. Diabetic Med. **14:** S1–S85.
2. CHOI, K.M., Y. ZHONG, B.D. HOIT, *et al*. 2002. Defective intracellular Ca(2+) signaling contributes to cardiomyopathy in type 1 diabetic rats. Am. J. Physiol. **283:** H1398–H1408.
3. HOWARTH, F.C., M.A. QURESHI & E. WHITE. 2002. Effects of hyperosmotic shrinking on ventricular myocyte shortening and intracellular Ca(2+) in streptozotocin-induced diabetic rats. Pflugers Arch. **444:** 446–451.
4. ISHIKAWA, T., H. KAJIWARA & S. KURIHARA. 1999. Alterations in contractile properties and $Ca2^{+}$ handling in streptozotocin-induced diabetic rat myocardium. Am. J. Physiol. **277:** H2185–H2194.
5. HOWARTH, F.C., S.C. CALAGHAN, M.R. BOYETT & E. WHITE. 1999. Effect of the microtubule polymerizing agent taxol on contraction, $Ca2^{+}$ transient and L-type $Ca2^{+}$ current in rat ventricular myocytes. J. Physiol. **516:** 409–419.
6. RUSKOAHO, H. 1992. Atrial natriuretic peptide: synthesis, release, and metabolism. Pharmacol. Rev. **44:** 479–602.
7. HALL, C. 2004. Essential biochemistry and physiology of (NT-pro)BNP. Eur. J. Heart Fail. **6:** 257–260.
8. WALTHER, T., S. HERINGER-WALTHER, R. TSCHOPE, *et al*. 2000. Opposite regulation of brain and C-type natriuretic peptides in the streptozotocin-diabetic cardiopathy. J. Mol. Endocrinol. **24:** 391–395.
9. CHRISTOFFERSEN, C., J.P. GOETZE, E.D. BARTELS, *et al*. 2002. Chamber-dependent expression of brain natriuretic peptide and its mRNA in normal and diabetic pig heart. Hypertension **40:** 54–60.
10. RUZICSKA, E., G. FOLDES, Z. LAKO-FUTO, *et al*. 2004. Cardiac gene expression of natriuretic substances is altered in streptozotocin-induced diabetes during angiotensin II-induced pressure overload. J. Hypertens. **22:** 1191–1200.
11. ROSENKRANZ, A.C., S.G. HOOD & R.L. WOODS. 2003. B-type natriuretic peptide prevents acute hypertrophic responses in the diabetic rat heart: importance of cyclic GMP. Diabetes **52:** 2389–2395.
12. CANDIDO, R., J.M. FORBES, M.C. THOMAS, *et al*. 2003. A breaker of advanced glycation end products attenuates diabetes-induced myocardial structural changes. Circ. Res. **92:** 785–792.
13. HOWARTH, F.C., A. ADEM, E.A. ADEGHATE, *et al*. 2005. Distribution of atrial natriuretic peptide and its effects on contraction and intracellular calcium in ventricular myocytes from streptozotocin-induced diabetic rat. Peptides **26:** 691–700.
14. HOWARTH, F.C., M. JACOBSON, O. NASEER & E. ADEGHATE. 2005. Short-term effects of streptozotocin-induced diabetes on the electrocardiogram, physical activity and body temperature in rats. Exp. Physiol. **90:** 237–245.