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Chronic nitrates blunt the effects of not only nitric oxide but also natriuretic peptides in cardiac myocytes

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Abstract

exposure to nitrates causes tachyphylaxis to nitric oxide (NO), which reduces the effects of the second messenger cyclic GMP. We tested the hypothesis that prolonged exposure to NO would also blunt the effects of natriuretic peptides. Cardiac myocytes were isolated from control (N=7) and chronic nitroglycerin (patched, N=7) rabbits. Patched animals received a transdermal nitroglycerin patch (0.3 mg/hr for 5 days). Myocyte function was determined at baseline, after CNP (C-type natriuretic peptide, 10^{-8} and 10^{-7} M) or BNP (Brain natriuretic peptide, 10^{-8} and 10^{-7} M) or SNAP (S-nitroso-N-acetyl-penicillamine, a NO donor, 10^{-6} and 10^{-5} M) followed by KT5823 (a cyclic GMP protein kinase inhibitor, 10^{-6} M). Soluble and particulate guanylyl cyclase activities were measured in vitro and phosphoprotein analysis was performed. In control animals, CNP 10^{-8} M ($5.14 \pm 0.5\%$) and 10^{-7} M ($4.4 \pm 0.7\%$) significantly reduced percentage shortening from baseline ($6.1 \pm 1.6\%$). KT5823 restored percentage shortening to $4.9 \pm 0.8\%$. Similar data were obtained with BNP and SNAP. In patched animals, CNP, BNP, SNAP had no significant effects on percentage shortening. The data on maximal rate of shortening and relaxation were consistent with these results. Guanylyl cyclase activities were not different in the control and patched animals. The myocytes from control and patched animals had similar protein phosphorylation patterns. Our data suggested that in addition to NO, the responses to both natriuretic peptides were downregulated after chronic exposure to nitroglycerin, but these effects were not due to changes in either guanylyl cyclase or cyclic GMP protein kinase, suggesting an altered downstream pathway.

Keywords

Nitroglycerin patch; Nitric oxide; Natriuretic peptides; Cardiac myocyte function; Rabbit

Introduction

Nitroglycerin and related organic nitrates are used in the treatment of cardiac ischemia. Cellular effects of nitroglycerin include activation of soluble guanylyl cyclase, which increases cyclic guanosine-3',-5'-monophosphate (cyclic GMP) levels and activates cyclic GMP-dependent

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protein kinases, cyclic GMP-regulated phosphodiesterases and cyclic nucleotide-gated ion channels [1]. Cyclic GMP usually exerts negative metabolic and functional effects in myocardium as well as in isolated cardiac myocytes [2]. Nitroglycerin exerts many of its biological effects via the release of nitric oxide (NO), which can also act through other mechanisms. The reaction of NO with cysteine residues, a process known as nitrosylation, is one important mechanism. S-nitrosylation is a redox signaling mechanism and that can regulate a broad range of biologic, physiologic and cellular functions [3].

The term 'nitrate tolerance' means that after prolonged, continuous or high dose nitrate treatment, its clinical effectiveness is attenuated. The underlying mechanism of tolerance has been studied extensively and several hypotheses have been proposed. Nitrate tolerance or tachyphylaxis is characterized by a reduction in the nitrate-induced cyclic GMP-mediated pathway [4]. Proposed mechanisms of tolerance include: (1) depletion of critical sulfhydryl groups [5], (2) desensitization of guanylyl cyclase [6], (3) impaired bioconversion of nitrates leading to diminished NO-release [7], (4) reduced generation of NO from nitrates [8], (5) increased activity of the cyclic GMP phosphodiesterase, which decreases cyclic GMP levels [9] and (6) a free radical hypothesis proposing increased oxidant stress [10,11]. Nitroglycerin-induced reactive oxygen species also inhibit the activation of nitroglycerin by thiol oxidation of mitochondrial aldehyde dehydrogenase which might explain why nitroglycerin elicits mitochondrial superoxide formation and nitrate tolerance [12].

Natriuretic peptides affect cardiovascular homeostasis primarily through blood volume and pressure regulation. Their anti-ischemic profile makes natriuretic peptides candidates for investigation as a potential treatment in myocardial ischemia [13]. The biological effects of natriuretic peptides are mediated by binding of the peptides to membrane-associated guanylyl cyclase receptors. There are two main subtypes (NPR-A and NPR-B) that mediate most effects of natriuretic peptides. Both receptors function as particulate guanylyl cyclases and elevate cyclic GMP. This is distinct from the soluble guanylyl cyclase stimulated by NO. Therefore both NO and natriuretic peptides produce cyclic GMP and act on a common cyclic GMP downstream pathway.

Previous studies have shown that chronic nitrates blunt the effects of NO. In the present study, we tested the hypothesis that prolonged exposure to nitrates would also blunt the effects of natriuretic peptides and examined whether this was related to the cyclic GMP pathway. Using a nitroglycerin patch to establish a nitrate tolerance animal model, we measured functional parameters of ventricular myocytes from control and patched rabbits to study the contractile changes in response to CNP (C- type natriuretic peptide), BNP (brain natriuretic peptide) or S-nitroso-N-acetyl-penicillamine (SNAP, a NO donor). We also examined downstream cyclic GMP signaling.

Methods

Animals and chemicals

All animals used in this study were maintained in accordance with the Guide for the Care of Laboratory Animals (DHHS Publication No. 85-23, revised 1996) and the study was approved by our Institutional Animal Care and Use Committee. All chemicals were obtained from Sigma Chemical Co., St. Louis, MO, unless otherwise stated.

Experimental preparation

Fourteen New Zealand white rabbits (2–3 kg) were divided into two groups: [1] Control (n=7), and [2] Chronic nitroglycerin (patched, n=7). For the patched group, the rabbits were tranquilized with subcutaneous injection of acepromazine maleate (5 mg). A transdermal

nitroglycerin patch (0.3 mg/hr for 5 days) was applied to the skin on the back after shaving the skin. The nitroglycerin patch was changed every morning for 4 days.

Myocyte isolation

Freshly isolated ventricular myocytes from both control and patched animals were routinely prepared by a standard protocol as described [14]. The animals were anesthetized with an overdose of sodium pentobarbital (90 mg/kg) followed by the administration of heparin (10 units/gram body weight) via intravenous injection using the circumflex ear vein. The heart was rapidly removed and the aorta was cannulated. Then the heart was subsequently mounted on a Langendorff perfusion apparatus. Aortic retrograde perfusion was initiated at 70 mm Hg constant pressure with modified minimal essential medium (MEM) supplemented with 10 mM taurine, 2 mM L-glutamic acid, and 20 mM HEPES, pH 7.20. After five minutes, the heart was perfused with MEM containing 0.1% type II collagenase (Worthington, Freehold, NJ) for 10 min. The perfusion buffer was maintained at 37°C and equilibrated with a water-saturated gas mixture (95% O₂, 5% CO₂). After collagenase digestion, the heart was removed from the apparatus and the ventricles were cut into small pieces. The tissue was subsequently pipetted in the 0.1% collagenase solution until all the large pieces were dispersed in the cell suspension. The supernatant containing isolated myocytes was then transferred to 15 ml centrifuge tubes. After washing once in MEM supplemented with 0.5% bovine serum albumin (BSA), 1 mM calcium was reintroduced in the presence of 10 mM 2, 3-butanedione monoxime. After being washed three times, the myocytes were suspended in the media supplemented with 0.5% BSA and 1 mM CaCl₂ at room temperature. Cell viability was assessed by a standard of maintenance of rod-shaped morphology and routinely was about 50–70%.

Measurements of Cardiac Myocyte Contractile Function

Details of the analysis of myocyte function have been reported previously [15]. Briefly, isolated cardiac myocytes were put into an open chamber on the stage of an inverted microscope (Zeiss Axiovert 125) in 2 ml of MEM solution supplemented with 0.5% BSA and 2 mM CaCl₂. Two platinum wires placed in the chamber were used to pace the myocytes (1 Hz, 5 msec duration, voltage 10% above threshold, and polarity alternated with each pulse). The chamber was maintained at 37°C throughout the measurement. The contraction of individual myocytes was measured with a Myotrack system containing a camera and a video edge detector (Crystal Biotech, Patton Biomedical). The data were collected continuously. The output of the video edge detector was fed into both a television monitor and a desktop computer, which was used to analyze the data. After a 5-min stabilization period, contraction data for the individual ventricular myocytes were recorded for a minimum of 10 consecutive contractions. We used at least three myocytes per protocol per experiment. The contraction data were continuously obtained. The parameters obtained during a single contraction included the diastolic cell length, percentage shortening, maximal rate of relaxation, maximal rate of shortening, time to peak shortening, time to 50% relaxation and time to 90% relaxation.

Measurements of Myocyte Guanylyl Cyclase Activity

The activity of soluble guanylyl cyclase from isolated ventricular myocytes was evaluated with a modification of the assay used by Sadoff et al [16]. A reaction system was used with total volume of 0.2 ml containing 50 mM Tris-HCl (pH 7.6), 10 mM theophylline, and a GTP regeneration system that contained 10 mM creatine phosphate, 10 µg creatine phosphokinase (200 U/mg protein), 4 mM MgCl₂, 1 mM GTP, in the absence or presence of 0.1 mM SNAP. The dose of SNAP could activate the enzyme maximally. Reactions were initiated by the addition of the cell extract and maintained at 37°C for 10 min. The reaction was terminated by the addition of sodium acetate (0.8 ml, 50 mM, pH 4.0) and the tube was placed in a water-bath at 90°C for 3 min.

Particulate guanylyl cyclase activity was measured based on the protocol used by Agullo et al¹⁵ with modifications. The GTP regeneration system was set up in a similar manner, however, 0.1% Triton X-100, was added to the solution containing the particulate fraction to elicit increased particulate guanylyl cyclase activity [17]. The reaction was maintained at 37°C for 10 min and later terminated by addition of 1 ml cold ethanol. The amount of cyclic GMP produced by enzyme activity was determined by radioimmunoassay.

In Vitro Phosphorylation Reaction and Phosphoprotein Analysis of Myocytes

All reactions were carried out in microfuge tubes at room temperature. Myocytes were obtained from both control (n=5) and patched (n=5) New Zealand White rabbits. All myocytes were homogenized (Polytron Homogenizer, 15 sec at 20,000 rpm) in buffer (5 mM Tris HCl, pH 7.4, 1 Mm MgCl₂, 0.25 M sucrose) and centrifuged at 15,500 rpm for 20 min at 4°C. The supernatant was divided and used as the myocyte extract for all phosphorylation reactions. An activator (8-Bromo-cGMP, 2.5×10^{-4} M) and an inhibitor of cyclic GMP protein kinase (KT5823, 7.5×10^{-4} M) was added to the 10 ml extract (0.5 mg total protein per ml) from both control and patched myocytes. Ten min were allowed for the reactants to equilibrate. After equilibration, each reaction tube was cooled on ice. Then 0.5 μ l [γ -P³³] ATP at 10 μ Ci/ml (Amersham) was added to initiate the reaction. The reaction was terminated 15 min later by adding a volume of Bio Rad reducing sample buffer equal to the entire reaction volume. The samples were heated at 95°C for 5 min and electrophoresed using miniature 12% SDS-polyacrylamide slab gels. The gels were then stained with Coomassie brilliant blue, destained overnight, dried using gel drier (Bio Rad) and exposed to X-ray film at 22°C for 36 hr. The exposed X-ray film demonstrated phosphate labeled proteins that were then sized by comparison to molecular weight standard markers.

Experimental Protocol

For both control and patched animals, three groups of myocytes were used in the following protocols for cell functional measurements. In all groups, myocytes were suspended in a well oxygenated chamber with 2 mM Ca²⁺-MEM at an appropriate concentration and the cells were allowed to stabilize for 10–15 min. The myocytes were paced with electrical field stimulation. A stabilization period of five minutes was used before the contraction data for the individual ventricular myocytes were recorded. A five-minute interval was allowed between reagent treatments and 10 consecutive contractions were used for analysis. For these three groups of myocytes, after the baseline recording, CNP (10^{-8} and 10^{-7} M) or BNP (10^{-8} and 10^{-7} M) or SNAP (10^{-6} and 10^{-5} M) followed by KT5823 (10^{-6} M) was added to determine the changes of myocyte function. Seven animals per treatment group were used and each experimental protocol was repeated so that at least 3 cells per protocol per animal were examined. Individual myocytes were required to complete their protocol for inclusion in the data. Untreated cells would continue to contract with a constant shortening level over the time course of these experiments. Results per protocol were averaged for analysis in each animal [2,16].

Statistical Analysis

A repeated measure analysis of variance (ANOVA) was used to compare variables measured in the experiment. Duncan's multiple range test was used to compare differences between baseline and the various treatments and groups. A value of $P < 0.05$ was used as the level of statistical significance. All values are expressed as mean $\pm$ S.E.M.

Results

Cell contraction measurements of both control and patched groups are presented in Figure 1 and Table 1. There was no statistical difference in baseline percentage shortening between the

control and patched groups, figure 1. There were also no differences in baseline maximum rate of shortening and relaxation between the control and patched groups, Table 1.

Figure 1 shows the effects of BNP, CNP and SNAP on percentage shortening in freshly isolated ventricular myocytes from control and patched rabbits. When BNP (10^{-8} and 10^{-7} M) was added to the control myocytes, percentage shortening was significantly decreased in a concentration dependent pattern by 20% and 30% (Fig. 1). After addition of KT5823, the percentage shortening rose slightly, but still was significantly below baseline. In the patched group, BNP did not significantly decrease percentage shortening. In the control group, when CNP (10^{-8} and 10^{-7} M) was added to the cells, percentage shortening decreased in a dose-dependent manner by 15% and 27%. At the higher dose of CNP (10^{-7} M), percentage shortening was significantly lower than baseline, but after addition of the KT5823, percentage shortening returned toward baseline. In the patched group, CNP did not significantly decrease percentage shortening. For both of doses (10^{-8} and 10^{-7} M) of CNP and KT5823, the percentage shortening of patched group myocytes was statistically higher than that of the control myocytes and was not different from baseline. When SNAP (10^{-6} and 10^{-5} M) was added to the control myocytes, the percentage shortening also decreased in a dose-dependent manner by 14% and 21%. The high dose of SNAP (10^{-5} M) significantly lowered percentage shortening. After addition of KT5823, the percentage shortening was reversed toward baseline. In the patched group, the effect of the SNAP on percentage shortening was blunted. After addition of the SNAP, there was no significant decrease of percentage shortening observed compared with the baseline. At the high dose of SNAP (10^{-5} M), the percentage shortening of patched group myocytes was significantly higher than that of the control myocytes.

Maximal rate of the shortening ($\mu\text{m}/\text{sec}$) and maximal rate of relaxation ($\mu\text{m}/\text{sec}$) were also examined. The high dose of CNP (10^{-7} M), BNP (10^{-7} M) and SNAP (10^{-5} M) significantly reduced the maximal rate of shortening when compared with the baseline. BNP (10^{-7} M) and SNAP (10^{-5} M) also significantly reduced the maximal rate of relaxation. While in the patched group, these agents did not significantly change maximum rate of shortening or relaxation (Table 1).

We determined whether the differential effects of nitric oxide or natriuretic peptides on myocyte function between the control and patched rabbits were due to alterations in guanylyl cyclase activity. Both particulate and soluble guanylyl cyclase activity were determined at baseline and after stimulation (Fig. 2). After stimulation with 0.1% Triton X-100, the enzymatic activity of particulate guanylyl cyclase from the control and patched groups was significantly increased from 4.9 ± 0.8 to 24.1 ± 3.1 pmol/mg protein/min and from 5.9 ± 0.6 to 25.3 ± 3.4 pmol/mg protein/min respectively. There were no differences between the control and patched groups at baseline or after stimulation. To examine soluble guanylyl cyclase activity, 0.1 mM SNAP was added to the control and patched groups, activity increased from 10.9 ± 1.5 to 88.9 ± 10.4 pmol/mg protein/min and from 10.2 ± 1.6 to 93.5 ± 16.1 pmol/mg protein/min respectively. No difference was observed between the control and patched groups at baseline or after stimulation.

To further study the effects of the cyclic GMP-dependent pathway on ventricular myocytes from control and patched rabbits, we performed in vitro phosphorylation and phosphoprotein analysis. Before stimulation with 8-Br-cGMP, both the control and patched group had a similar basal level of protein phosphorylation (Fig. 3, lane 1 and lane 4). After addition of 8-Br-cGMP, there were several bands at approximate molecular masses of 170, 130, 97, 62 and 14 kDa that were significantly enhanced in the control myocytes (lane 2). With simultaneous addition of KT5823 (lane 3), the intensities of bands were reduced, indicating that cyclic GMP-dependent protein phosphorylation occurred. When compared with the control group, the overall

phosphorylation pattern of patched group and the degree of phosphorylation of these proteins caused by cyclic GMP were similar.

Discussion

Chronic exposure to nitrates leads to a blunting of their effects on the heart. The major finding of this study was that the effects of natriuretic peptides on cardiac myocytes were also blunted by chronic high dose administration of nitrates. We found that after long-term exposure to nitrates, the effects of both natriuretic peptides and NO were reduced. Natriuretic peptides and nitric oxide reduced myocyte function and after chronic nitroglycerin this effect was blunted. We examined the downstream pathway and found that neither particulate nor soluble guanylyl cyclase was affected by chronic nitroglycerin. In addition, the effects cyclic GMP protein kinase and its ability to phosphorylate proteins were not changed. This indicated that the downregulation of the functional effects of natriuretic peptides after chronic nitroglycerin was not directly due to changes in the traditional cyclic GMP-dependent pathway.

Nitric oxide exerts many of its cellular effects through activation of soluble guanylyl cyclase, which increases cyclic GMP levels and activates cyclic GMP-dependent protein kinases, cyclic GMP-regulated phosphodiesterases and cyclic nucleotide-gated ion channels [1]. Cyclic GMP usually exerts negative metabolic and functional effects in myocardium as well as in isolated cardiac myocytes [2]. Nitroglycerin, commonly used in the treatment of coronary artery and ischemic heart disease, acts through the release of NO. Many of its additional responses are mediated through the covalent attachment of NO to either cysteines or transition metals, a process known as nitrosylation. Nitrosylation is a physiologically important post-translational modification that affects a wide variety of proteins involved in a number of cellular processes. The term of nitrosative stress implied the hazardous levels of nitrosylation, a situation analogous to and taking place in the presence of oxidative stress which have been implicated in cardiac apoptosis, muscle dysfunction and cell injury [18–20]. NO also acts through soluble guanylyl cyclase and activates this enzyme to raise cyclic GMP levels.

Natriuretic peptides also lead to increased production of cyclic GMP. The principal natriuretic peptides are atrial natriuretic peptides (ANP), brain natriuretic peptides (BNP) and C-type natriuretic peptides (CNP), though other members of the natriuretic peptides family also exist. Natriuretic peptides function through the membrane-associated receptors with associated particulate guanylyl cyclase domains that can elevate the intracellular level of cyclic GMP. The two subtypes of these receptors (NPR-A and NPR-B) that elevate cyclic GMP are widely expressed in the cardiovascular system [21]. Elevation of intracellular cyclic GMP concentration can be demonstrated in all tissues and cell types expressing NPR-A or NPR-B following exposure to natriuretic peptides [21].

We found that both BNP and CNP, acting through NPR-A and NPR-B respectively, produced a significant reduction in cardiac myocyte function. This was demonstrated by significant reductions in percentage shortening and maximal rates of shortening and relaxation of the rabbit cardiac myocytes. Similar effects on the heart have been demonstrated previously [10,13,14]. Inhibition of the cyclic GMP protein kinase with KT5823 partially reversed these effects. This suggested that part of the negative functional effects of the natriuretic peptides were related to activation of this protein kinase.

Long-term administration of nitrates causes nitrate tolerance or tachyphylaxis in many species including man [4,5,7,8,10,11]. Recently, there has been a strong focus on a proposed free radical hypothesis. Munzel et al [22] demonstrated increased superoxide production in vessels exposed to nitrate. Superoxide can be generated via multiple systems, including xanthine oxidase, NADH/NADPH oxidase and nitric-oxide synthase. The elevation of superoxide can

limit the bioavailability of both endogenous and exogenous NO through the diffusion controlled reaction of superoxide with NO to produce peroxynitrite [23,24]. Peroxynitrite is an oxidizing and nitrating cytotoxin that could contribute to nitrate tolerance through interference with the normal function of nitric oxide synthase or the cyclic GMP-dependent protein kinase [25,26].

Nitrate tolerance reduces the responses to NO through the second messenger cyclic GMP, but it was not clear whether the natriuretic peptide-cyclic GMP signaling pathway would also be affected. In the present study, we observed significant decrements in the effects of natriuretic peptides on cardiac myocytes after nitrite tolerance had been induced. Most studies of nitrate tolerance have focused on vascular smooth muscle myocytes while we investigated this aspect using functional contractile ventricular myocytes. We found that the effects of activation of both the particulate and soluble guanylyl cyclase by natriuretic peptides and NO were reduced after chronic nitroglycerin.

Pretreatment with a nitroglycerin in rabbits was used to establish nitrate tolerance. This model has been used previously [8]. Similar to our previous studies [15,27], we observed that SNAP, a NO donor inhibited myocyte function in a dose-dependent manner. Natriuretic peptides have been reported to have diverse effects on cardiac function in different models [13,21]. Our current data demonstrating functional decrements were consistent with other reports [28,29]. After chronic nitroglycerin administration, these effects of natriuretic peptides and nitric oxide were greatly reduced.

We examined some of the potential mechanisms for these diminished effects of NO and natriuretic peptides. The reduced effects of CNP, BNP and SNAP were not due to alterations in either soluble or particulate guanylyl cyclases. Neither the basal nor stimulated activities of these cyclases were markedly changed by chronic nitroglycerin, fig. 3. In addition, the lack of effects of natriuretic peptides after chronic nitroglycerin could not be related to proposed reductions in NO release or generation [7,8].

Many of the functional effects of CNP, BNP and SNAP in ventricular myocytes are related to downstream effectors in the cyclic GMP signal transduction pathway, such as the cyclic GMP protein kinase. We observed that KT5823, a cyclic GMP protein kinase inhibitor, could partially reverse the negative effects of CNP, BNP and SNAP on control myocyte contraction. With pretreatment using the nitroglycerin patch, all effects of the cyclic GMP system were attenuated including effects of KT5823. However, the in vitro phosphorylation assay demonstrated no changes. The overall phosphorylation reaction pattern of patched group was similar to the control group. Thus, both the guanylyl cyclases and the cyclic GMP dependent protein kinase appear unaffected by chronic exposure to nitroglycerin. This suggests that other cyclic GMP downstream effects or molecules outside of this signaling pathway may be altered in nitrate tolerant myocytes. These could be proteins affected by nitrosylation or free radical formation [3,22–24]. This study indicated that nitrate tolerance blunted the effects of natriuretic peptides, but not directly through the cyclic GMP signaling system. This result suggests that downstream cyclic GMP effectors were desensitized or there were other changes in this pathway, perhaps related to free radical formation or protein nitrosylation. Further research is required to understand the precise mechanism.

In summary, the present study demonstrated that after chronic high dose exposure to nitrates, not only were the effects of functional actions of nitric oxide blunted, but the effects of natriuretic peptides were also diminished. We showed that this downregulated response to natriuretic peptides after chronic exposure to nitroglycerin was not due to changes in particulate guanylyl cyclase activity or to alterations in cyclic GMP protein kinase activity. These data suggest that there were other changes in the downstream cyclic GMP pathway or changes in proteins or mechanisms outside of this pathway during nitrate tolerance.

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Figure 1.

Effects of CNP (Top), BNP (Middle) and SNAP (Bottom) on percentage shortening in freshly isolated ventricular myocytes from control and patched rabbits. Data are shown as mean $\pm$ S.E.M. (N=7). *P<0.05 vs. baseline, +P<0.05 vs. control rabbits.

Figure 2. Particulate and soluble guanylyl cyclase activity in ventricular myocytes from control and patched rabbits at baseline and after stimulation with 0.1% Triton X-100 (particulate) and 0.1 mM SNAP (soluble) (N=5).

Figure 3.

Effects of cyclic GMP-dependent protein phosphorylation on ventricular myocytes from control (lanes 1–3) and patched (lanes 4–6) groups of rabbits. An equal amount of protein (5 μ g) was loaded on each lane. Lanes 1 and 4 show the basal activities for protein phosphorylation of the two groups. Lane 2 and 5 show protein phosphorylation after addition (+) of 8-Br-cGMP. Lane 3 and 6 show phosphorylation in the presence of both 8-Br-cGMP and the cyclic GMP protein kinase inhibitor KT5823. Molecular weights standards are shown on the left. Note the increased phosphorylation with 8-Br-cGMP and the effect of KT5823 in reducing the degree of phosphorylation. This result indicated that the myocytes of the control and patched animals had a similar pattern of cyclic GMP-dependent protein phosphorylation

Table 1

The effects of CNP, BNP and SNAP on maximal rate of shortening and maximal rate of relaxation in ventricular myocytes from control and patched rabbits hearts

	Maximal Rate of Shortening ($\mu\text{m/s}$)		Maximal Rate of Relaxation ($\mu\text{m/s}$)	
	Control	Patched	Control	Patched
Baseline	70.7 $\pm$ 7.3	70.4 $\pm$ 2.8	68.2 $\pm$ 6.5	67.2 $\pm$ 2.9
CNP				
10^{-8} M	62.2 $\pm$ 6.3	67.2 $\pm$ 2.6	61.6 $\pm$ 6.0	64.4 $\pm$ 2.5
10^{-7} M	54.8 $\pm$ 4.4 *	63.1 $\pm$ 2.5	54.0 $\pm$ 4.1	61.2 $\pm$ 2.1
+KT5823, 10^{-6} M	59.5 $\pm$ 6.1	60.6 $\pm$ 2.7	58.9 $\pm$ 5.5	59.0 $\pm$ 2.7
Baseline	64.9 $\pm$ 4.6	61.8 $\pm$ 5.5	64.7 $\pm$ 3.9	59.0 $\pm$ 5.7
BNP				
10^{-8} M	55.6 $\pm$ 3.5	61.8 $\pm$ 5.2	54.0 $\pm$ 3.6	59.3 $\pm$ 4.8
10^{-7} M	49.2 $\pm$ 3.4 *	60.0 $\pm$ 6.0	48.3 $\pm$ 3.9 *	58.0 $\pm$ 5.6
+KT5823, 10^{-6} M	56.2 $\pm$ 4.4	61.2 $\pm$ 5.8	54.3 $\pm$ 4.3	60.4 $\pm$ 6.3
Baseline	71.7 $\pm$ 6.8	70.1 $\pm$ 3.0	68.7 $\pm$ 6.2	67.2 $\pm$ 3.0
SNAP				
10^{-6} M	60.0 $\pm$ 5.6	67.5 $\pm$ 3.0	58.2 $\pm$ 4.7	64.6 $\pm$ 3.2
10^{-5} M	57.0 $\pm$ 4.1 *	65.6 $\pm$ 3.5	54.6 $\pm$ 3.6 *	62.7 $\pm$ 3.4
+KT5823, 10^{-6} M	58.7 $\pm$ 5.3	65.9 $\pm$ 3.2	55.7 $\pm$ 5.1	64.3 $\pm$ 3.7

Note: values are means $\pm$ SEM.

* significantly different from baseline.