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C-type natriuretic peptide has a negative inotropic effect on cardiac myocytes

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Abstract

C-type natriuretic peptide (CNP) has vasodilatory and antimitogenic actions, but its role in the control of cardiac function is unclear. We studied the effect of CNP on cultured, beating neonatal rat cardiac myocytes. CNP caused a significant reduction in the amplitude of contraction and a significant accumulation of intracellular cyclic GMP. The effect of a membrane permeable cyclic GMP on cell contraction was similar to that of CNP. CNP caused no change in Ca^{2+} transients. Blockade of natriuretic peptide receptors abolished the effects of CNP on contraction and accumulation of intracellular cyclic GMP. Blockade of cyclic GMP-dependent protein kinase abolished the effect of CNP on myocyte contraction. We conclude that CNP has a negative inotropic effect on neonatal rat cardiac myocytes. The effect of CNP is mediated via natriuretic peptide receptor(s) causing elevation of intracellular cyclic GMP which possibly activates protein kinase and causes attenuation of myofilament sensitivity to Ca^{2+}.

Keywords: Cell culture; Contractile function; Myocyte; Natriuretic peptide; Protein kinase

1. Introduction

C-type natriuretic peptide (CNP), the third member of the natriuretic peptide family, is a 22-amino acid peptide with structural homology to atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP). While first discovered in porcine brain (Sudoh et al., 1990), CNP was later shown to be produced by, and secreted from endothelial cells (Stingo et al., 1992; Suga et al., 1992). Unlike the hormonal nature of ANP and BNP, CNP is believed to have a more paracrine role and to exert its action on the neighboring cells. CNP has higher affinity to the B-type natriuretic peptide receptor (NPR_B) while ANP and BNP bind to the A-type receptor (NPR_A) (Espiner et al., 1995). Like ANP and BNP, CNP has vasodilatory and antimitogenic actions; however, it lacks the diuretic and natriuretic properties of the other two peptides (Cao and Gardner., 1995; Charles et al., 1995).

CNP is present in cardiac tissue, and elevated concentrations of CNP were found in patients with congestive heart failure (Wei et al., 1993), However, the role of CNP in the heart and the direct effects of CNP on cardiac myocytes are still unclear.

Our objective was to determine the effect of CNP on contraction of neonatal rat cardiac myocyte and to define the intracellular mechanism of its action.

2. Materials and methods

2.1. Cell culture

Myocardial cells from ventricle fragments of hearts of 1-day-old Sabra rats were isolated by serial trypsinizations as described (Hallaq et al., 1989). Cells were suspended in F-10 medium (Bet Haemek) containing 20% heat inactivated fetal calf serum and penicillin–streptomycin–gentamycin antibiotic solution. The cell suspensions were made myocyte-rich by preplating on tissue culture plastic Petri dishes for 30 min to allow attachment of fibroblasts to the Petri dish (Blondel et al., 1971). The myocyte-enriched suspension was collected and diluted with the same medium to 5×10^5 cells/ml. The cells were plated on

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Petri dishes containing circular cover slips for contractility measurements, and without cover slips for cGMP measurements. Cells were maintained in humidified 5% CO_2 95% air atmosphere at 37°C for 3–5 days until studied. At the time of the experiment, the cells had reached a confluent monolayer consisting of 85–90% myocytes which exhibited spontaneous contractions (Ela et al., 1993).

2.2. Myocyte contraction

Amplitude of systolic motion was measured by a video-motion system using an inverted microscope (Nikon) as described (Barry and Smith, 1982; Fixler et al., 1994). In brief, coverslips with contracting myocytes were placed in a specially designed chamber which was maintained at a constant temperature of 37°C. Cells were constantly superfused with buffered salt solution (BSS) containing (mM): NaCl 140, KCl 5, $CaCl_2$ 1, $MgCl_2$ 1, glucose 10, Na_2HPO_4 1, HEPES 10, pH 7.4. Myocytes were field-stimulated about 25% above threshold with 2.0 ms square wave pulses (30–40 V) through two platinum electrodes based in the superfusion solution, and connected to a stimulator (Grass Instruments, MA, USA), and were paced at 2 Hz. Cell motion was assessed by video-motion detector (video-motion analyzer 633 CVI, Colorado Video, USA) measuring the motion of a single cellular marker. Data were then displayed on an oscilloscope (E for M, USA), recorded and stored for further analysis. In addition to the amplitude of contraction, the rate of contraction and relaxation (dl/dt) was measured. CNP (Phoenix Pharmaceuticals, Mountain View, CA) at different concentrations was added to the superfusion solution followed by a washout period. Recording was performed at baseline, at different time intervals during CNP infusion and at washout. Each experiment consisted of one dose only. The amplitude of cell movement was determined as the mean of six successive tracings during each time period.

2.3. Cyclic GMP production

The level of intracellular cyclic GMP, the putative second messenger of CNP in myocytes, was measured by radioimmunoassay. Cell cultures were prepared as described. Cells were incubated on 30-mm Petri dishes in BSS at 37°C in room atmosphere for 1 h. First, cyclic GMP was measured following different incubation periods with CNP to determine time-dependent cyclic GMP accumulation, and the plateau reached at 15 min. Thus, agents were added to the BSS for 15 min incubation. 3-Isobutyl-1-methylxanthine (IBMX, 0.05 mM) was also added to inhibit degradation of cyclic GMP. Incubation was stopped by aspirating the medium and the addition of 0.25 ml EDTA (4 mM, pH 7.5), to prevent enzymatic degradation of cyclic GMP. Cells were then gently scraped, collected and heated for 3 min to coagulate proteins. The suspension was centrifuged and cyclic GMP in the supernatant was assessed by a cyclic [^{3}H]GMP assay system (TRK 500, Amersham International, UK). In order to confirm that the source of the cyclic GMP measured from the myocyte-rich culture is the myocytes [comprising 85–90% of the cells in the culture (Ela et al., 1993)], cyclic GMP accumulation of the myocyte-rich culture was compared to that of a fibroblast-rich culture.

2.4. Intracellular Ca^{2+} transients

Intracellular Ca^{2+} transients were measured using the fluorescent calcium indicator indo-1/acetoxymethylester (Indo-1/AM) (molecular probes). The indo-1 loading solution was composed of BSS, probenecid (3 mM) and Indo-1/AM (6 μM). Cultures were incubated in the loading solution for 45 min at room temperature in the dark and then transferred to a similar solution without indo-1 for an additional 15 min. The coverslips with the loaded cardiomyocytes were then placed in the cell chamber. Fluorescence was read with an FM-100 dual wavelength (410/480 nm) fluorescence microphotometer (Ricon, San Paulo, CA) connected to an inverted microscope. Autofluorescence of unloaded cultures was determined and subtracted from the measurements of indo-1 loaded cells before computations of the ratio. Measurements were done before and at different time intervals after addition of CNP (10^{-7} M). Between measurements, cells were maintained in the dark to minimize indo-1 bleaching (Hallaq et al., 1989). Since some degree of bleaching occurred, the change of indo 1-fluorescence ratio of cells treated with CNP was compared to that of cells perfused with BSS alone (time control). Time control was performed on the same day and in the same cell culture as the treatment study. Results have not been calibrated to $[Ca^{2+}]i$ values, but are presented as indo-1 fluorescence ratios.

2.5. Statistical analysis

Results are reported as means ± standard error. Comparison of baseline and treatment data was done using Student's paired *t*-test. Comparison between two groups was done using Student's unpaired *t*-test. Comparison between more than two groups (different concentrations) or repeated measurements was done using ANOVA. $p < 0.05$ was considered significant.

3. Results

3.1. Myocyte contraction

Fig. 1 shows time and dose–response to CNP in cultured, beating neonatal rat cardiac myocytes. Perfusion of cardiac myocytes with CNP (10^{-7} M) resulted in a reduction in contraction amplitude that reached maximal effect after 10 min perfusion (Fig. 1a). Fig. 1b displays the

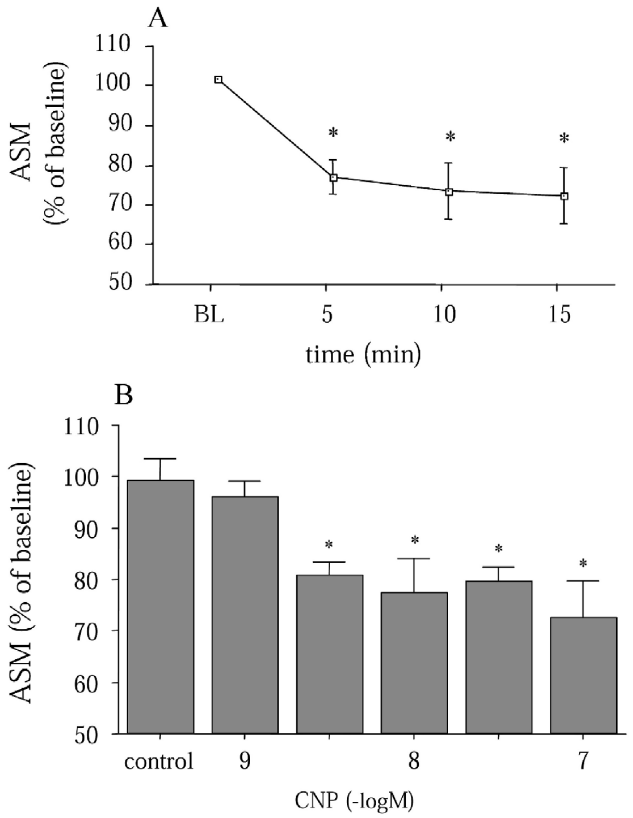

Fig. 1. The effect of CNP on contraction in neonatal rat cardiac myocyte. Cultured neonatal rat myocytes were paced at 2 Hz. Amplitude of systolic motion (ASM) was measured by video-motion system. For each experiment, steady baseline served as control. (A) The amplitude of systolic motion is expressed as % of baseline (BL) at different time intervals during CNP (10^{-7} M) perfusion. $^{*}p < 0.05$ vs. baseline (ANOVA), $n = 5$. (B) The effect of different doses of CNP on amplitude of systolic motion following 10 min perfusion, in neonatal rat cardiac myocyte. Displayed is the amplitude of systolic motion expressed as % of control without CNP (control) and with the addition of CNP at 10^{-9} to 10^{-7} M. $^{*}p < 0.05$ vs. control for each experiment (ANOVA), $n = 5$–8.

contraction amplitude of the myocytes following 10 min BSS or CNP perfusion at different concentrations. Cells perfused with BSS alone and those perfused with CNP at 10^{-9} M showed no change in contraction amplitude. Contraction amplitude was significantly reduced in cells perfused with CNP at doses ranging from 5×10^{-9} to 10^{-7} M. Partial recovery of contraction amplitude was obtained by a 10-min washout (no shown). The negative inotropic effect induced by CNP displayed a threshold dose dependency since no significant decrease was observed following the increase in CNP concentrations from 5×10^{-9} to 10^{-7} M. The greatest effect observed was reduction of contraction amplitude to 65% of baseline after 15-min infusion with CNP at 10^{-7} M, just before recovery. The rates of contraction and relaxation (dl/dt) were also reduced by CNP. At 10^{-7} M, CNP caused a reduction in the rate of contraction to $81 \pm 4\%$ of baseline ($p < 0.05$) and the rate of relaxation to $80 \pm 4\%$ of baseline ($p < 0.05$).

3.2. CNP receptor(s) inhibition

A natriuretic peptide receptor (NPR_A and NPR_B) antagonist, HS-142-1 (Zhang et al., 1994), was used. Following steady baseline recording, myocytes were perfused with CNP (10^{-7} M) for 10 min. HS-142-1 (2.5×10^{-6} M) was then added to the perfusate for a 10-min period of co-perfusion. The dose of CNP used was that which exerted the greatest negative inotropic effect, as described above. The dose of HS-142-1 was chosen according to published data (Zhang et al., 1994) and dose–response studies at our laboratory. Myocyte contraction amplitude was assessed at baseline and following perfusion with CNP only and with CNP together with HS 142-1. The natriuretic peptide receptor blocker HS-142-1 (2.5×10^{-6} M) completely abolished the negative inotropic effect of CNP (10^{-7} M) on neonatal rat cardiac myocytes, as displayed in Fig. 2 [$p < 0.05$ for CNP vs. baseline ($n = 28$) and vs. CNP + HS-142-1 ($n = 10$); $p = $ NS for baseline vs. HS-142-1]. When cells were pretreated with HS-142-1, administration of CNP (10^{-7} M) had no inotropic effect ($98.1 \pm 6.1\%$ vs. HS 142-1 alone, $p = $ NS, $n = 8$).

3.3. Cyclic GMP production

As shown in Fig. 3, incubation of neonatal rat myocytes with CNP at 10^{-7} M in the presence of IBMX resulted in accumulation of intracellular cyclic GMP. Cyclic GMP levels, after 15-min incubation, were 22 ± 8.25 pmol/mg protein in the control group, and 264 ± 99 pmol/ mg protein in myocytes treated with CNP ($p < 0.01$). Pretreatment of the myocytes with HS-142-1 resulted in a dose-de-

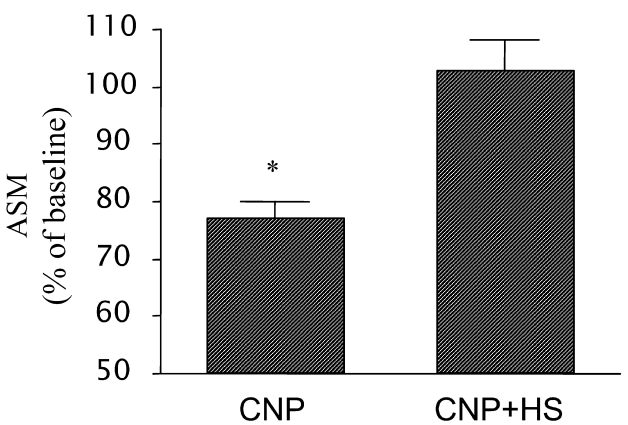

Fig. 2. The effect of receptor blockade on amplitude of systolic motion (ASM). Cultured neonatal rat myocytes were paced at 2 Hz. ASM was measured as described and displayed as % of steady baseline. After 10-min perfusion with CNP (10^{-7} M, $n = 28$), a significant reduction in amplitude of systolic motion was observed (CNP). The addition of the natriuretic peptide receptor blocker HS-142-1 (2.5×10^{-6} M) to cells treated for 10 min with CNP resulted in the complete reversal of the effect of CNP on amplitude of systolic motion (CNP + HS, $n = 10$). $^{*}p < 0.05$ vs. baseline and CNP + HS (Student's t-test).

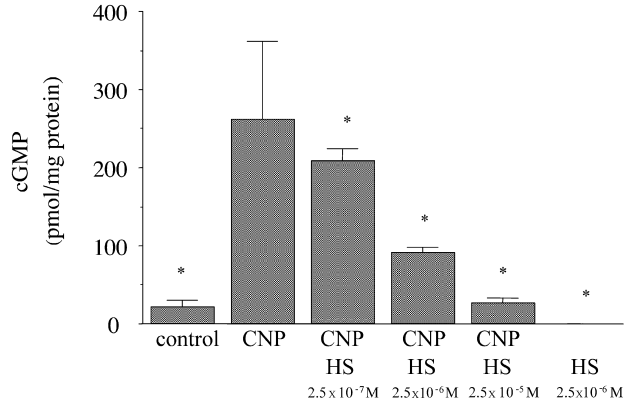

Fig. 3. CNP effect on intracellular cGMP. The effect of CNP (10^{-7} M) alone and together with the natriuretic peptide receptor blocker, HS-142-1, at different doses, on intracellular cyclic GMP in neonatal rat cardiac myocytes. Intracellular cyclic GMP was measured by radioimmoassay. Following a 15-min incubation, CNP caused significant accumulation of intracellular cyclic GMP. HS 142-1 alone had no effect on cyclic GMP accumulation (HS 2.5×10^{-6} M, the right column), while it dose dependently inhibited CNP-induced cyclic GMP accumulation and abolished it at 2.5×10^{-5} M ($^{*}p<0.05$ vs. CNP, ANOVA). The inhibitory effect of HS-142-1 at 2.5×10^{-5} M on CNP-induced cyclic GMP accumulation was significantly greater than that of HS-142-1 at 2.5×10^{-7} M. Assays performed in triplicate.

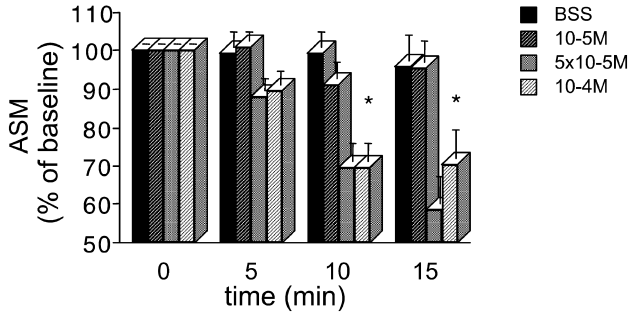

Fig. 4. The effect of membrane permeable cyclic GMP, 8-br-cGMP, at different concentrations on contraction. Cultured neonatal rat myocytes were paced at 2 Hz. ASM was measured by video-motion system. For each experiment, steady baseline served as control. The amplitude of systolic motion is expressed as % of control at different intervals during 8-br-cGMP perfusion. At each time interval, bars represent amplitude of systolic motion without 8-br-cGMP (BSS, far left) and with 8-br-cGMP doses 10^{-5}, 5×10^{-5} and 10^{-4} M on the far right. Each dose was a separate experiment. Data are means $\pm$ S.E.M. of 8–12 experiments for each dose. 8-br-cGMP at both 5×10^{-5} and 10^{-4} M doses significantly reduced the amplitude of systolic motion compared to BSS, steady baseline (time 0) and 10^{-5} M dose ($^{*}p<0.05$, ANOVA).

pendent inhibition of CNP-induced cGMP accumulation. HS-142-1 at 2.5×10^{-6} M significantly inhibited CNP-induced cGMP accumulation to 93.5 ± 6.0 pmol/ mg protein, while at 2.5×10^{-5} M it completely blocked CNP-induced cGMP accumulation (Fig. 3). Incubation of myocytes with HS-142-1 alone, at 2.5×10^{-6} M, resulted in no measurable cGMP accumulation. This was not significantly different from cGMP accumulation in the control cells. To determine whether the increase in cGMP in our myocyte-rich cultures did not originate from the small fraction of fibroblasts present, we prepared fibroblast-rich cultures. Incubation of fibroblast-rich culture with CNP (10^{-7} M) resulted in cyclic GMP accumulation which was 46% less than that observed in the myocyte-rich culture at the same dose of CNP, strongly suggesting that the main source of cyclic GMP in our pre-plated, myocyte-rich culture was cardiac myocytes.

3.4. Externally administered cyclic GMP

Fig. 4 shows the incubation of neonatal rat cardiac myocytes with a cell permeable cyclic GMP, 8-br-cGMP. At 10^{-5} M, 8-br-cGMP caused no significant reduction in contraction amplitude, while at 5×10^{-5} and 10^{-4} M it reduced contraction amplitude significantly. The effects of 5×10^{-5} and 10^{-4} M were not different. Contraction of control cells (BSS) was stable throughout the treatment period. The negative inotropic effect of 8-br-cGMP was similar to that of CNP in magnitude, time course and threshold effect. At 5×10^{-5} M, 8-br-cGMP reduced the rates of contraction and relaxation (dl/dt) to $72.3\pm4\%$, and $75.6\pm6\%$ of baseline, respectively. The effects of 8-br-cGMP on the rates of contraction and relaxation were similar to those of CNP.

3.5. Ca^{2+} transients and sensitivity to Ca^{2+}

Fig. 5 is a representative simultaneous recording of Ca^{2+} transients and cell contraction. The amplitude of Ca^{2+} transients, presented as a ratio, decreased slightly with time, due to a small bleaching effect of the indicator.

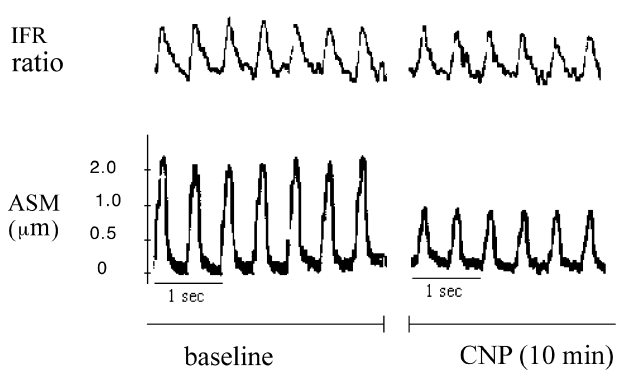

Fig. 5. The effect of CNP on amplitude of systolic motion and calcium transients. Representative tracings displaying simultaneous recording of amplitude of systolic motion (ASM) by video-motion analyzer, and calcium transients, measured in Indo-1/AM loaded myocytes, as described in Methods. Fluorescence values were not calibrated to $[Ca^{2+}]i$ values, but are presented as Indo-1 fluorescence ratios (IFR). The tracings are at steady baseline (baseline) and after a 10-min CNP (10^{-7} M) perfusion (CNP-10 min). The slight reduction with time in calcium transient amplitude was observed in the time course of the control, and is probably due to bleaching of the indicator.

This was evident in the time course of control and CNP-treated cells. Contraction amplitude, on the other hand decreased markedly following the addition of CNP. Fig. 6 shows data of simultaneous recording of Ca^{2+} transients and cell contraction. Compared to myocytes perfused with BSS alone ($n = 15$), myocytes perfused with CNP (10^{-7} M, $n = 8$) exhibited no significant change in Ca^{2+} transients ($96 \pm 3\%$ of control, $p =$ NS). Contraction amplitude, on the other hand, decreased significantly, as already shown, suggesting that the decrease in contractility induced by CNP is mainly due to a reduction in myofilament sensitivity to Ca^{2+}.

3.6. Cyclic GMP-dependent protein kinase inhibition

The results described above suggest that in neonatal rat cardiac myocytes exposure to CNP causes accumulation of cyclic GMP leading to a negative inotropic effect by reduction in the myofilament sensitivity to calcium. To examine whether this effect is mediated by cyclic GMP-dependent protein kinase, we examined the effect of cyclic GMP-dependent protein kinase inhibitor, KT 5823, on contractility in the presence and absence of CNP. KT 5823, at low concentrations, is considered to be a specific inhibitor of cyclic GMP-dependent protein kinase. The K_i of KT 5823 for cyclic GMP-dependent protein kinase was shown to be 0.234 μM and for a cyclic AMP-dependent protein kinase was more than 10 μM (Kase et al., 1987). Myocyte monolayers on coverslips were preincubated for 10 min with KT 5823 (1 μM). The effect of KT 5823 on contractility was determined. CNP (10^{-7} M) was then added to the perfusate and the effect of co-perfusion of CNP and KT 5823 on myocyte contractility was compared to the effect of CNP alone.

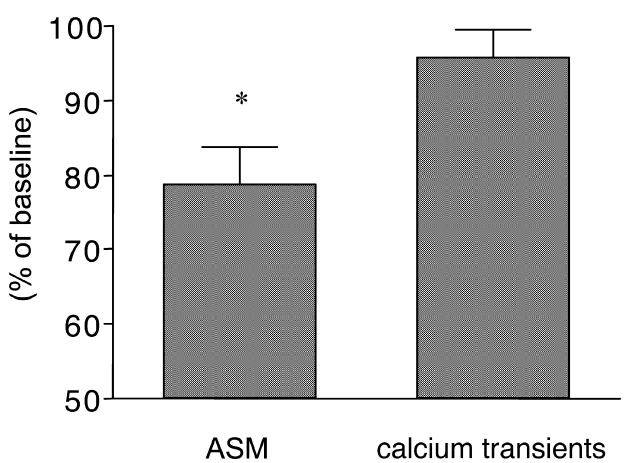

Fig. 6. The effect of CNP (10^{-7} M) on the amplitude of systolic motion and calcium transients. The effect of CNP on calcium transients was measured using the fluorescent calcium indicator Indo-1/AM, as described in Methods. The fluorescence values were not calibrated to $[Ca^{2+}]i$ values, but are presented as Indo-1 fluorescence ratios, because of the slight reduction in calcium transient amplitude in the time course of the control as well as CNP-perfused preparations. Indo 1-fluorescence ratios of myocytes perfused for 10 min with CNP (10^{-7} M, $n = 8$) were compared to those of myocytes perfused for 10 min with BSS alone ($n = 15$). ASM was measured by video-motion analyzer in the same cultured myocytes in which calcium transients were measured ($n = 8$). Contraction amplitude of the myocytes perfused for 10 min with CNP (10^{-7} M) was compared to steady baseline, as described. CNP caused significant reduction in the amplitude of contraction while it did not change Indo 1-fluorescence ratio. * $p < 0.05$ vs. baseline (Student's t-test).

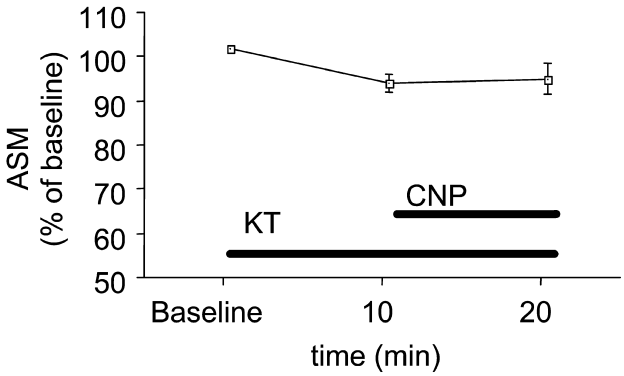

Fig. 7. The effect of inhibition of cyclic GMP-dependent protein kinase on amplitude of systolic motion. Neonatal rat cardiac myocytes were pretreated for 10 min with KT 5823 (1 μM, KT), a cyclic GMP-dependent protein kinase inhibitor. CNP was then added to the perfusate (CNP). ASM was measured as described ($n = 3$). Perfusion with KT 5823 alone resulted in no significant change in the amplitude of systolic motion. There was no change in the amplitude of systolic motion (ANOVA) following the addition of CNP (10^{-7} M) to the perfusion medium of cells perfused with 1 μM KT 5823.

As shown in Fig. 7, KT 5823 alone had no effect on contraction amplitude compared to baseline control ($92.3 \pm 2.1\%$ of baseline, $p =$ NS), but it completely inhibited the negative inotropic effect of CNP ($93.3 \pm 3.4\%$ of baseline, $p =$ NS vs. baseline and KT 5823, $n = 3$).

4. Discussion

CNP has been shown to be present in normal human coronary artery endothelium (Naruko et al., 1996) and its gene expression has been found in rat myocardial cells (Lin et al., 1995). CNP thus may participate in the control of cardiac function. Indeed, we have shown in this report that CNP has a negative inotropic effect on neonatal rat cardiac myocytes. The magnitude of the effect of CNP on contraction is comparable to that reported for nitric oxide (Brady et al., 1993). Reduction in contraction amplitude was associated with the elevation of intracellular cyclic GMP levels, and the inotropic effect of CNP was reproducible by externally administered cyclic GMP. This strongly suggests that the inotropic effect of CNP is mediated via cyclic GMP. Although serum levels of CNP are in the range of pmol/l (Tutsune et al., 1994), and we have observed inotropic effects in concentrations ranging from nmol/l, CNP is believed to be a paracrine factor secreted by the endothelial cell to act on the neighboring myocyte.

Thus, the myocyte may be exposed to concentrations of CNP in the range used in this study, and the plasma CNP may represent "spill-over" of the peptide with no significant endocrine effect.

Recent studies have reported both negative, positive or no inotropic effect for CNP in several species. In adult rat papillary muscles, Brusq et al. (1999) found positive lusotropic and negative inotropic effect. In canine-perfused atrial preparations, Hirose et al. (1998) and Beaulieu et al. (1997) reported positive inotropic effect. Shah et al. (1994) found no significant effect of ANP or CNP on twitched amplitude of adult rat cardiac myocytes. The possible reasons for the apparent contradictory results are: (1) in atrial preparations as well as papillary muscles, there are endothelial cells, fibroblasts, and other cell types in addition to myocytes. Thus, the effects observed could have been secondary to reactive changes rather than a pure effect of CNP on myocytes. (2) In the reports of Hirose et al. (1998) and Beaulieu et al. (1997), the inotropic effect was evident $\sim$1 min after a bolus injection of CNP. We have continuously infused the peptide and have observed a gradual elevation of cGMP and reduction in contractility starting about 2 min after the beginning of peptide infusion, reaching its maximal effect in 10 min. Thus, the effect of bolus injection may differ from that of continuous infusion. (3) There are differences in the species used, preparation of the tissue and the age of the animals, which may contribute to the different results. There are very few data on the direct effect of CNP on isolated cultured cardiac myocytes, and none on neonatal rat cardiomyocytes. Neonatal rat cardiomyocytes are different from adult rat cardiomyocytes in morphology, structure and response to several agents (Novakova et al., 1995).

We have found no significant change in Ca^{2+} transients following administration of CNP in neonatal rat myocytes. In accord with this concept is the recent report of Kaye et al. (1999) who found nitric oxide to exert a Ca^{2+}-independent negative inotropic effect in adult rat cardiac myocytes. Furthermore, ANP was found to inhibit an intracellular step necessary for Ca^{2+}-dependent stimulation of aldosterone biosynthesis in adrenal glomerulosa cells (Lotshaw et al., 1991) and Ca^{2+}-dependent stimulation of dopamine efflux in pheochromocytoma cells (Kanwal et al., 1997). The effect of ANP on Ca^{2+} channels is controversial. In frog ventricular myocytes, ANP decreased the Ca^{2+} channels current that had been increased by beta-adrenoreceptor stimulation, but did not affect the basal Ca^{2+} channels current (Gisbert and Fischmeister, 1988). In neonatal rat cardiac myocytes, McCall and Fried (1990) found ANP to decrease Ca^{2+} influx. Recently, Tohse et al. (1995) reported that in rabbit heart cells, human ANP as well as cyclic GMP produced a decrease in amplitude of the basal L-type Ca^{2+} channels current. A number of factors could account for these differences. The different receptors for the natriuretic peptides may have different effects on Ca^{2+} channels. NPR_B, the receptor with higher affinity for CNP may not affect Ca^{2+} channels, while activation of NPR_A, the receptor with higher affinity for ANP, may decrease Ca^{2+} channel currents. In addition, different species may have different responses to the natriuretic peptides.

Our results suggest that, as in other cell types (Espiner et al., 1995), CNP binds to a specific natriuretic peptide receptor, and activates a particular guanylate cyclase to convert GTP to cyclic GMP. It is of interest that the natriuretic peptide receptor antagonist, HS 142-1, at a concentration of 2.5×10^{-6} M abolished the inotropic effect of CNP but only partially inhibited the CNP-induced accumulation of intracellular cyclic GMP. Ten-fold increase in the concentration of HS 142-1 (2.5×10^{-5} M) completely abolished the CNP-induced accumulation of cyclic GMP. These data suggest that a small increase in the intracellular level of cGMP, such as in the presence of 2.5×10^{-6} M HS 142-1, may not cause a change in contraction amplitude.

Recent studies showed that, in isolated cardiac myocytes, cyclic GMP decreased contractility but improved relaxation. Furthermore, cyclic GMP abolished the diastolic dysfunction following brief hypoxia in cardiac myocytes (Shah et al., 1995). These data suggest an important role for cyclic GMP and cyclic GMP generating agents on cardiac function. In the present study, CNP, as well as externally administered cGMP (8-br-cGMP) caused a reduction in the rate of both contraction and relaxation. The magnitude of reduction in the rate of contraction and relaxation is similar to the magnitude of reduction in contraction amplitude, thus resulting in no significant change in the duration of contraction and relaxation. Different experimental conditions may be responsible for these differences.

We have demonstrated that the negative inotropic effect of CNP is not accompanied by a significant reduction in peak Ca^{2+} transients, suggesting that this effect is a result of attenuation of myofilament sensitivity to Ca^{2+}. KT 5823, an inhibitor of cyclic GMP-dependent protein kinase, abolished the effect of CNP on contraction amplitude. In their elegant report, Kaye et al. (1999) showed that the cGMP analog, 8-bromo-cGMP, significantly increased troponin I phosphorylation in adult rat cardiac myocytes. This effect was attenuated by KT 5823, suggesting that cyclic GMP activates a specific protein kinase which, possibly through other signaling proteins, phosphorylates troponin I. Troponin I phosphorylation has been related to reduction in myofilament sensitivity to Ca^{2+} (Kaye et al., 1999). Reduction in myofilament response to Ca^{2+} may also be due to reduction in intracellular pH, which was not measured in this study.

In conclusion, the present study demonstrates, for the first time, a negative inotropic effect of CNP on isolated neonatal rat myocytes. This study further shows that the effect of CNP is mediated via the natriuretic peptide receptor causing an elevation of intracellular cyclic GMP

which activates protein kinase and possibly causes attenuation of myofilament sensitivity to Ca^{2+}. These results suggest that CNP participates in the control of cardiac function in neonatal rats.

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