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Opposing effects of endothelin-1 on C-type natriuretic peptide actions in rat cardiomyocytes

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Abstract

C-type natriuretic peptide (CNP) and Endothelin-1 are paracrine peptides with opposing vascular and mitogenic actions. In cardiac myocytes, CNP reduced contractility and induced accumulation of cyclic guanosine monophosphate (cGMP). Endothelin-1 caused an increase in contractile amplitude, abolished the negative inotropic effect of CNP, reduced the negative inotropic effect of a membrane permeable cGMP, and inhibited cGMP accumulation induced by CNP. We conclude that endothelin-1 abolishes the negative inotropic effect of CNP. This effect may be mediated by inhibition of the negative inotropic actions of cGMP as well as by reduction of cGMP levels.

Keywords: Natriuretic peptide; Endothelin; Neonatal, rat; Myocyte; Culture; cGMP

1. Introduction

C-type natriuretic peptide (CNP) is the third member of the natriuretic peptide family. Like the other natriuretic peptides, it has vasodilatory and antimitogenic actions. CNP binds to a specific receptor, guanilate cyclase, and causes an increase in intracellular cyclic guanosine monophosphate (cGMP) (Espiner et al., 1995). We have recently shown that in cultured neonatal rat cardiac myocytes, CNP reduces myocyte contraction amplitude and myofilament responsiveness to calcium. This action is receptor specific, and mediated via cGMP and cGMP-dependent protein kinase (Nir et al., 2001). Endothelin-1 is a very potent vasoconstrictor and pro-mitogenic agent. Both CNP and endothelin-1 are secreted from endothelial cells, and are believed to act on the neighboring cells in a paracrine manner. Both peptides are present in cardiac tissue (Picard et al., 1998; Wei et al., 1993). A balance between the vasoconstrictor-mitogenic agents and the vasodilator-antimitogenic agents seems to play a role in the control of vascular tone and cardiac function. Congestive heart failure is characterized by elevated circulating and cardiac levels of both CNP and endothelin-1. The hemodynamic effects of the natriuretic peptides are thought to be beneficial in this disease state, however, these actions are blunted in overt congestive heart failure (Nakamura et al., 1998). The reason for this decline in the natriuretic peptide effect is thought to be, at least partially, due to the high concentrations of circulating and intracellular endothelin-1. Little information is available on the modulating effect of endothelin-1 on CNP actions. This study is aimed to investigate the effects of endothelin-1 on the inotropic and cGMP inducing actions of CNP in cultured myocytes.

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 and were made myocyte-rich by pre-plating on tissue culture plastic Petri dishes for 30 min to allow attachment of fibroblasts to the Petri dish. The myocyte enriched suspension was collected and diluted to 5×10^5 cells/ml.

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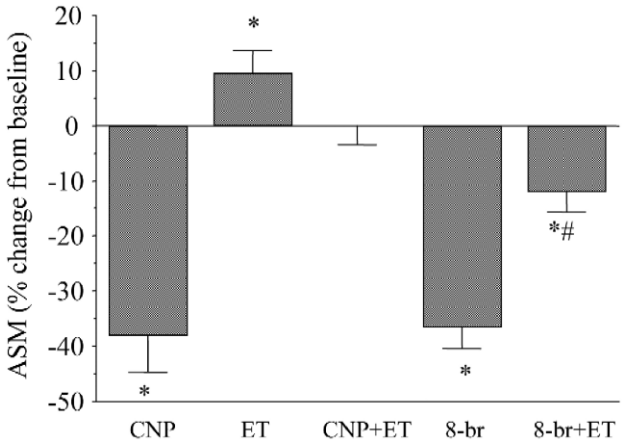

Fig. 1. The effect of CNP, endothelin-1 and 8-BrcGMP on neonatal rat cardiac myocyte contraction. Cultured neonatal rat cardiomyocytes were paced at 2 Hz. Amplitude of systolic motion (ASM) was measured by video-motion system. For each experiment, steady baseline served as control. The amplitude of systolic motion is expressed as % of control at maximal effect. Endothelin-1, when co-infused, was added 5 min after the addition of CNP or 8-BrcGMP. Each bar represents a separate experiment. CNP (10^{-7} mol/l), endothelin-1 (ET, 10^{-7} mol/l), 8-BrcGMP (8-br, 10^{-5} mol/l). Data are means ± S.E.M. of five to eight experiments. $^{*}P < 0.05$ vs. control.

The cells were plated on 35-mm Petri dishes, and 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 have reached a confluent monolayer consisting of 85–90% myocytes, which exhibit spontaneous contractions (Ela et al., 1993).

2.2. Myocyte contraction

The cultured cells were paced at 2 Hz. Contraction amplitude was measured by a video-motion system using an inverted microscope (Nikon) as described (Fixler et al., 1994). Cell motion was assessed by video-motion detector (video-motion analyzer 633 CVI Colorado Video USA) measuring the motion of a single cellular marker. Every experiment consisted of one dose only. The amplitude of cell movement was determined as the mean of six successive tracings during each time period. The peptides and treatment agents (as detailed under Section 2.3) were added to the superfusion solution. In the relevant experiments, endothelin-1 was added to cells superfused with CNP or 8-BrcGMP for 5 min, and both agents were co-administered for 15 min. This was compared to the effect of CNP alone.

2.3. Peptides and drugs

We have previously shown a dose dependent effect of CNP on myocyte contraction amplitude (Nir et al., 2001) with the greatest effect at 10^{-7} mol/l. Thus, CNP (Sigma) at this dose was used in all experiments. Endothelin-1 (Sigma) was superfused at different doses ranging from 10^{-7} to 10^{-9} mol/l. Cyclic GMP analog, 8-BrcGMP (Sigma) at 5×10^{-5} mol/l, a dose that resulted in effects similar to CNP 10^{-7} mol/l, was used. A natriuretic peptide receptor (type A and type B) antagonist, HS-142-1 (Zhang et al., 1994) was used.

2.4. Cyclic GMP content

The level of intracellular cGMP, the putative second messenger of CNP in myocytes, was measured by radioimmunoassay. Cells were incubated in Petri dishes for 15 min with buffered salt solution (BSS) or the different agents and cGMP levels measured. The effect of endothelin-1 on CNP-induced cGMP accumulation was assessed by pre-incubation with endothelin-1 at different doses. Three-isobutyl-1-methylxanthine (IBMX, Sigma, 0.5 m mol/l) was added to inhibit degradation of cGMP. Incubation was stopped by aspirating the medium and addition of 0.25 ml EDTA (4 m mol/l, pH 7.5), to prevent enzymatic degradation of cGMP. Cells were then gently scraped, collected and heated for 3 min to coagulate proteins. The suspension was centrifugated and cGMP in the supernatant was assessed by cyclic GMP [^{3}H] assay system (TRK 500, Amersham International UK).

2.5. Statistical analysis

Results are reported as mean ± standard error. Comparison of baseline and treatment data was done using Stu-

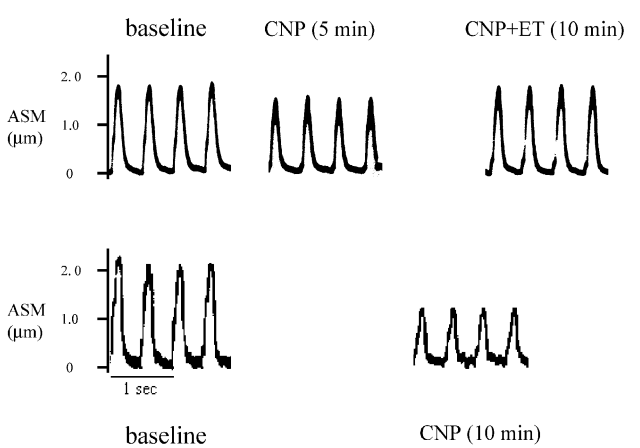

Fig. 2. Representative tracings: the effect of CNP on neonatal rat cardiomyocyte contraction and the modulating effect of endothelin-1 on CNP actions. Cultured neonatal rat myocytes were paced at 2 Hz. Amplitude of systolic motion (ASM) was measured by video-motion system. The bottom tracing shows myocyte contraction at baseline and following 10 min infusion with CNP (10^{-7} mol/l, CNP 10 min). Significant reduction of ASM is evident. The top tracing shows myocyte contraction at baseline, following 5 min infusion with CNP (10^{-7} mol/l, CNP 5 min), and following the addition of endothelin-1 (10^{-7} mol/l), which was then co-infused for 10 min with CNP (CNP + ET, 10 min). While ASM started to decline after 5 min of CNP treatment, the addition of endothelin-1 reversed the inotropic effect of CNP.

dent's paired *t*-test. Comparison between two groups was done using Student's unpaired *t*-test. Comparison between more than two groups or repeated measurements were done using analysis of variance (ANOVA). $P < 0.05$ was considered significant.

3. Results

As shown previously, superfusion of neonatal rat cardiac myocytes with CNP (10^{-7} mol/l) resulted in a significant reduction in amplitude of contraction by 37% from the baseline contraction amplitude (Figs. 1 and 2). Incubation of myocytes with CNP (10^{-7} mol/l) resulted in accumulation of intracellular cGMP to 239 ± 10 pmol/mg protein ($n = 18$). The natriuretic peptide receptor antagonist, HS-142-1, at 2.5×10^{-5} mol/l, completely abolished CNP-induced cGMP accumulation (28 ± 0.9 pmol/mg protein, $n = 3$). Endothelin-1, at the same concentration as CNP (10^{-7} mol/l), caused a significant, small, increase in contraction amplitude to 109% of baseline (Fig. 1). The inotropic effects of both peptides returned to baseline level with cessation of peptide administration. The addition of endothelin-1 to cells treated with CNP abolished the effect of CNP on myocyte contraction amplitude (Figs. 1 and 2). The effect of endothelin-1 on CNP-induced cGMP accumulation was time dependent with the greatest effect observed after 20-min pre-incubation. Endothelin-1 dose dependently reduced, but did not abolish the CNP-induced cGMP accumulation (Fig. 3). Endothelin-1 alone had no effect on intracellular cGMP (Fig. 3). These results indicate that the antagonistic effect of endothelin-1 on contractility is mediated via a reduction of CNP-induced cGMP accumulation. However, while endothelin-1 abolished the inotropic effect of CNP (Figs. 1 and 2), it only inhibited intracellular cGMP accumulation by 58% (Fig. 3), suggesting an additional site for inhibition of the inotropic effect. Thus, the effect of endothelin-1 on the inotropic effect of membrane permeable cGMP, 8-BrcGMP, was assessed. While endothelin-1 had no effect on 8-br-cGMP-induced cGMP immunoreactivity (Fig. 3), it significantly reduced the effect of 8-BrcGMP on contraction (Fig. 1).

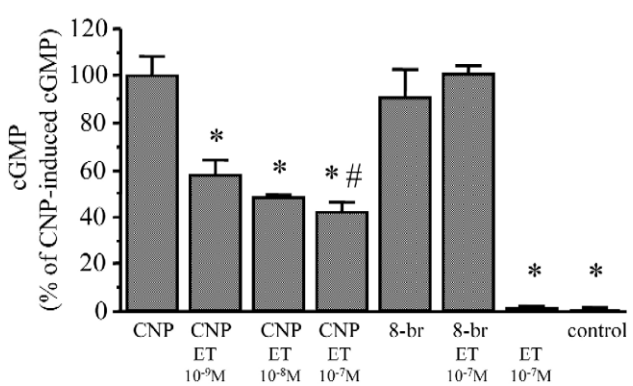

Fig. 3. The effect of endothelin-1 on the levels of intracellular cGMP. Cultured neonatal rat cardiomyocytes were prepared as described. Cells were incubated for 15 min with the different agents. Cyclic GMP levels were measured by radioimmunoassay as described. Endothelin-1 preincubation was performed for 20 min prior to the addition of CNP or 8-BrcGMP. Cyclic GMP levels are expressed as % of CNP induced levels. CNP (10^{-7} mol/l), 8-BrcGMP (8-br, 10^{-5} mol/l), endothelin-1 (ET); $n = 3$; $^{*}P < 0.05$ vs. CNP, $\#P < 0.05$ vs. endothelin-1 10^{-9} mol/l.

4. Discussion

Our previous study demonstrated that CNP reduces contraction amplitude in cultured neonatal rat cardiac myocytes (Nir et al., 2001). In the present study, we have found that endothelin-1 has a small, positive inotropic effect, and when administered following CNP, abolished the negative inotropic effect of the latter. Endothelin-1 also inhibited the negative inotropic effect of the membrane permeable cGMP analog, 8-BrcGMP, and attenuated the CNP-induced accumulation of intracellular cGMP. It is, thus, suggested that the modulating effect of endothelin-1 on CNP-induced inotropic effect is due to inhibition of both cGMP accumulation, and the inotropic effect of cGMP. Endothelin-1 had no effect on cGMP immunoreactivity induced by 8-BrcGMP. As 8-BrcGMP may be less degradable than native cGMP, even in the presence of IBMX, the question whether the effect of endothelin-1 on cGMP accumulation is due to increased degradation or reduced formation remains to be determined.

The interaction of endothelin-1 with the natriuretic peptides has been reported in several studied. Hanehira et al. (1997) showed that in cultured vascular smooth muscle cells, angiotensin II and arginine vassopressin-induced endothelin-1 release, was inhibited by the natriuretic peptides. CNP was the most potent inhibitor (CNP $\gg$ ANP $>$ BNP), and 8-BrcGMP had similar effects. While endothelin-1 increases ANP and BNP production and/or release in atrial myocytes (Leskinen et al., 1997), it inhibits their cGMP generating effect. In a very elegant study, Vigne et al. (1994) showed that in brain capillary endothelial cells, endothelin-1 inhibited CNP induced cGMP generation, an effect comparable to what we found in cardiac myocytes. They have also found that the effect of endothelin-1 is not mediated through increasing cGMP breakdown. The modulating effect of endothelin-1 on contractile amplitude, and cGMP generating action of CNP in cardiac myocytes has not been reported previously.

Endothelin-1 inhibited the inotropic effect of 8-BrcGMP. This is a very important observation, which has not been reported in myocytes. It suggests that in addition to inhibition of cGMP accumulation, endothelin-1 antagonizes the biological actions of cGMP. Very little has been reported on the effect of endothelin-1 on the intracellular actions of

cGMP. Suenobu et al. (1999) examined the effect of cGMP producing agents (nitric oxide and the natriuretic peptides) and endothelin-1 on apoptosis in rat vascular endothelial cells. They reported that cGMP-producing agents as well as 8-BrcGMP induced apoptosis, whereas, endothelin-1 was anti-apoptotic. Similar to the results presented in our study, endothelin-1 inhibited the effect of 8-BrcGMP on apoptosis. The mechanism for this phenomenon is not clear, but the authors speculated that the effect of cGMP is mediated via protein kinase G and that endothelin-1 counteracts this effect. We (Nir et al., 2001) have shown that in neonatal rat cardiomyocytes, CNP, via protein kinase G reduces myofilament responsiveness to calcium. Endothelin-1, may, in a yet undefined way counteract this effect. Further studies to determine the mechanism of the effect of endothelin-1 on CNP and other cGMP-inducing agents are needed.

In conclusion, this study shows that endothelin-1 abolishes the effect of CNP on neonatal rat cardiac myocyte contraction. Inhibition of the negative inotropic actions of cGMP as well as reduction of cGMP level may contribute to the effect of endothelin-1.

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