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Interactions between endothelin-1 and atrial natriuretic peptide influence cultured chick cardiac myocyte contractility

Yvonnick Bézie [a], Laurence Mesnard [b], Dan Longrois [a], Françoise Samson [b], Claudine Perret [a], Jean-Jacques Mercadier [b], Stéphane Laurent [a,b,*]

[a] *Laboratoire de Pharmacologie et INSERM U 337, Faculté de Médecine Broussais Hôtel-Dieu, Paris, France*
[b] *Département de Cardiologie Moléculaire et Cellulaire, C.N.R.S. URA 1159, Le Plessis-Robinson, France*

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Abstract

We have previously shown that rat atrial natriuretic peptide (ANP) reduces the contractility of cultured, spontaneously beating chick embryo ventricular cells, an effect opposite to that of endothelin-1. Endothelin-1 has been described as a secretagogue for natriuretic peptides in vitro and in vivo. Natriuretic peptides can inhibit endothelin-1 secretion from cultured endothelial cells, suggesting a negative feedback mechanism between endothelial cells and cardiomyocytes. The aim of this study was to determine whether ANP attenuated the endothelin-1-induced increase in myocyte contractility. Using a video-microscopy system we studied the contractility of isolated cultured chick ventricular myocytes in response to endothelin-1, chicken natriuretic peptide (ChNP), and both. We also used Northern blot analysis to study the time course of ChNP expression in response to endothelin-1. Endothelin-1 (10^{-8} M) increased chick cardiomyocyte contractility by 20–25% between 5 and 15 min ($P < 0.05$). Although ChNP (3×10^{-7} M) did not significantly change the amplitude of contraction in basal conditions, it prevented the endothelin-1-induced increase in contractility ($P < 0.05$) when perfused prior to endothelin-1, and reversed it when perfused 5 min after endothelin-1 exposure ($P < 0.05$). Endothelin-1 significantly increased the accumulation of ChNP mRNA in chick ventricular myocytes as early as the 30 min after exposure ($P < 0.05$), with a maximal effect after 2 h of stimulation ($P < 0.01$); no effect was observed after 4 h. These data support an interaction between endothelin-1 and natriuretic peptides as autocrine/paracrine factors regulating the contractile function of chick cardiac myocytes, as well as their antagonistic effects on cardiac cell contractility. The early and transient expression of ChNP mRNA in response to endothelin-1 may be involved in this interaction.

Keywords: Cardiac myocyte, cultured; Cardiac contractility; Endothelin-1; ANP (atrial natriuretic peptide), chicken; ChNP (chicken natriuretic peptide) mRNA

1. Introduction

Local factors released by the endocardium and/or microvascular endothelium have been shown to regulate the contractile function of cardiac myocytes (Brutsaert et al., 1988; Ishikawa et al., 1988; Shah and Lewis, 1993). One major candidate for mediating paracrine effects in the myocardium is endothelin-1 (Ishikawa et al., 1988). Endothelin-1 has been widely reported to increase cardiac contractility, both in vivo and in vitro (Ishikawa et al., 1988; Concas et al., 1989; Rubanyi and Polokoff, 1994). Cardiac natriuretic peptides regulate salt and water homeostasis, as well as blood pressure (Brenner et al., 1990). The main in vivo stimulus of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) secretion by myocytes appears to be mechanical stretching of the atrial wall (Ruskoaho, 1992). Other stimuli, like endothelin-1, can also affect ANP secretion. Endothelin-1 (10^{-10}–10^{-7} M) stimulates ANP secretion in basal conditions in cultured atrial cardiomyocytes (Gardner et al., 1991; Sei and Glembotski, 1990) and perfused isolated rat atrial tissues (Hu et al., 1988), and after stretching in isolated rat hearts (Mantymaa et al., 1990). Endothelin-1 has been described as a secretagogue for ANP through an endothelin ET_A-like receptor (Thibault et al., 1994). BNP secretion is also stimulated by endothelin-1 (Suzuki et al., 1992; Horio et al., 1992, Horio et al., 1993), while natriuretic peptides can

* Corresponding author. Département de Pharmacologie et INSERM U 337, Faculté de Médecine Broussais Hôtel-Dieu, 15 Rue de l'Ecole de Médecine, 75670 Paris cedex 06, France. Tel.: 33 1 43 95 91 00; fax: 33 1 43 95 81 00.

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influence the secretion of endothelin-1. Indeed, ANP and BNP have been reported to inhibit endothelin-1 secretion from cultured endothelial cells in basal and/or stimulated states (Kohno et al., 1991, 1992; Hu et al., 1992). These results point to a negative feedback mechanism between endothelial cells and cardiomyocytes, endothelin-1-enhancing natriuretic peptide secretion by myocytes, and natriuretic peptides attenuating endothelin-1 secretion by endothelial cells (Kohno et al., 1991, 1992; Horio et al., 1992; Hu et al., 1992; Suzuki et al., 1992). The involvement of this paracrine interaction in the regulation of cardiomyocyte contractility has not yet been studied.

The inotropic effect of natriuretic peptides has been less extensively studied than that of endothelin-1. We have shown that rat ANP can reduce the contractility of cultured, spontaneously beating chick embryo ventricular cells (Vaxelaire et al., 1989). The inotropic effect of ANP in vivo is controversial (Seymour et al., 1985; Kleinert et al., 1986; Shapiro et al., 1986). However, Rankin and Swift (1990) recently reported that ANP has a negative inotropic action in the anesthetized rabbit. Taken together, these results suggest that endothelin-1 could attenuate its own inotropic effect through an ANP-mediated decrease in contractility. We thus investigated the antagonistic actions of endothelin-1 and natriuretic peptides on cardiomyocyte contractility. We studied the contractility of isolated chick ventricular myocytes in response to endothelin-1 and/or chicken natriuretic peptide (ChNP) in a validated model of cultured chick ventricular myocytes (Laurent et al., 1985; Briand et al., 1989; Vaxelaire et al., 1989). To further document the effects of endothelin-1 on ChNP expression by chick cardiac myocytes, we used the same model to study the time course of ChNP mRNA accumulation following endothelin-1 stimulation.

2. Materials and methods

2.1. Cell culture

Monolayer cultures of spontaneously beating chick embryo ventricular cells were prepared as previously described (Laurent et al., 1985; Briand et al., 1989; Vaxelaire et al., 1989) with minor modifications. Briefly, 11-day-old chick embryo hearts were removed in sterile conditions and the ventricles were cut into small fragments and placed in culture in M 199 medium containing Hanks' salt, 10% new-born calf serum, glutamine (200 mM), penicillin (100 IU/ml), streptomycin (100 μg/ml) and sodium bicarbonate. The ventricular fragments were gently agitated in 10 ml of 0.08% (w/v) trypsin in phosphate-buffered saline (PBS) lacking Ca^{2+} and Mg^{2+} at 37°C for 7 cycles of 7 min each. Trypsinization was then blocked by adding 10 ml of cold culture medium. The cell suspension was centrifuged at $1500 \times g$ for 10 min, the supernatant phase was decanted and the cells were resuspended in culture medium. Final concentrations (in mM) in the culture medium were Na^+ 144, Ca^{2+} 0.97, K^+ 4.0, HCO_3^- 18, Mg^{2+} 0.8 and Cl^- 131. After elimination of nonmuscle cells by differential adhesion, the myocyte suspension was diluted to 3×10^5 cells/ml and placed in plastic Petri tissue-culture dishes. For the measurement of contractility, rectangular glass coverslips were placed at the bottom of the Petri dishes prior to cell seeding. Cultures were incubated in a humidified atmosphere of 5% CO_2 in air at 37°C. For the measurement of myocyte contractility, culture medium was replaced after 24 h of incubation by Hanks' solution supplemented with glutamine, antibiotics and 5% newborn calf serum. For the determination of ChNP mRNA accumulation, medium was removed after 24 h and replaced by the same medium in which newborn calf serum was replaced by insulin, transferrin and sodium selenite (10 μg/ml each). All studies were performed on 3- to 4-day cultures.

2.2. Measurement of contractility

Cardiocyte motion was observed and recorded with an electro-optical system (Barry and Smith, 1982; Laurent et al., 1985; Vaxelaire et al., 1989; Yin et al., 1994). A glass coverslip covered with a chick ventricular myocyte monolayer was placed in a chamber provided with inlet and exit ports for culture perfusion. The chamber was placed on the stage of an inverted phase-contrast microscope (Leitz Diavert) connected to a video camera (CCCTV Hitachi) and the image of contracting cells was displayed on a video monitor; the total magnification of the image on the monitor screen was ×1625. A photovoltaic cell with an aperture diameter of 5 mm was positioned on the video screen over one edge of a beating heart-cell wall and the change in voltage output, corresponding to the amplitude of cell wall motion, was recorded continuously on a channel of a Gould-Brush 3302 recorder. As cell wall motion varied with the position and portion of the cell being monitored, only the relative amplitude of wall motion was measured. The beating rate was calculated from the recording of cell wall motion. Cells were electrically driven via a pair of platinized electrodes, and cathodic square pulses of 20 ms and up to 60 mA were used for stimulation. The medium bathing the cells before and after superfusion of test substances was medium 199 containing 1.2 mM Ca^{2+} at pH 7.4. After a 15-min equilibrium period, the cells were first superfused with a solution containing a Ca^{2+} concentration (3.6 mM) that elicited the maximal inotropic response (Concas et al., 1989; Vaxelaire et al., 1989). When the response had stabilized (10 min), the monolayer was superfused with test solutions after a washout period of 5 min. For each cell studied, results were expressed as the percentage increase in the amplitude of contraction, as compared to baseline. We tested the effect of ChNP (3×10^{-7} M) and endothelin-1 (10^{-8} M) on cardiomyocyte contractility by superfusing the coverslip with each test

solution for 15 min. These concentrations of ChNP and endothelin-1 were selected from published concentration-effect curves (Concas et al., 1989; Vaxelaire et al., 1989). Interactions between ChNP and endothelin-1 on myocyte contractility were studied by superfusing cell monolayers with endothelin-1 (10^{-8} M) for 5 min, then adding ChNP (3.10^{-7} M) or control culture medium to the perfusate for 10 min. In another set of experiments, ChNP and control solutions were perfused for 10 min before adding endothelin-1 for 5 min.

2.3. RNA purification and Northern blot analysis

Cultured chick embryo ventricular cells were incubated with or without endothelin-1 (10^{-8} M) for 15 min, 30 min, and 1, 2, 4, 18 and 24 h. At least three experiments with two different cell cultures were performed for the short stimulations (from 15 min to 4 h). RNA was extracted from cultured cells by using the acid guanidinium thiocyanate-phenol-chloroform extraction method (Chomczinsky and Sacchi, 1987). RNA purity and concentration were determined by spectrophotometry at 260 and 280 nm, and 5 μg of total RNA were size-fractionated by means of agarose gel electrophoresis and transferred onto a nylon membrane (Hybond N^+, Amersham). Deposition of similar amounts of RNA in each lane was confirmed by visual examination of ribosomal RNA after ethidium bromide staining. After RNA fixation by ultraviolet cross-linking (0.15 J/cm2) the membrane was prehybridized with Rapid Hybridization Buffer (Amersham) at 55°C for 1 h. Hybridization was carried out at 65°C for 2 h using a full-length ChNP cDNA probe (Akisuki et al., 1991; Mesnard et al., 1993) radiolabeled with ^{32}P by primer extension from the linearized plasmid, or at 55°C for 2 h using a 24-mer synthetic oligonucleotide complementary to rat 18S RNA and end-labeled using T4 polynucleotide kinase. The filters were washed in appropriate conditions of stringency and the membranes were exposed to X-ray film with intensifying screens at −70°C. ChNP mRNA accumulation was quantified by densitometry of the corresponding autoradiograms (VERNON PHI), and normalized to the hybridization signal obtained with the 18S RNA probe to correct for differences in loading and/or transfer efficiencies.

2.4. Data analysis and statistics

The contractile response was analyzed after analysis of serial measurements (Mathiews et al., 1990), in which the area under the curve of the time-dependent contractile effect (A.U.C.) was considered as a parameter. The mean ± S.D. A.U.C. for each group of cells were compared using Student's unpaired *t*-test or a one-way analysis of variance (ANOVA), depending on the number of groups to be compared.

The effects of the test agents on ChNP expression were assessed by two-way analysis of variance. Results are presented as means ± S.E.M.; differences with P values of < 0.05 were considered significant.

2.5. Materials

ChNP (chicken atrial natriuretic peptide) and human-porcine endothelin-1 were from Sigma (France); M 199 culture medium and new-born calf serum were from Eurobio (France); insulin, transferrin and sodium selenite media supplement were from Sigma (France), and porcine trypsin was from Biosys (France).

3. Results

3.1. Effects of endothelin-1 and ChNP on cardiomyocyte contractility

Following 10 min of cell superfusion with Ca^{2+} (3.6 mM), the increase in cardiomyocyte contractility (+37.9 ± 2.4%) was similar in the different cultures, reflecting their good quality (data not shown).

As shown in Fig. 1, endothelin-1 (10^{-8} M) increased the mean amplitude of cell contraction by 20–25% between 5 and 15 min ($P < 0.05$), in good agreement with our previous results (Concas et al., 1989). Surprisingly, ChNP (3×10^{-7} M) did not attenuate the contractility of myocytes in basal conditions (Fig. 1). This contrasted with the effect of rat ANP on myocyte contractility in the same model (Vaxelaire et al., 1989).

In contrast, ChNP had marked effects on myocyte contractility when it was perfused before or after endothelin-1. Indeed, ChNP reversed the endothelin-1-induced

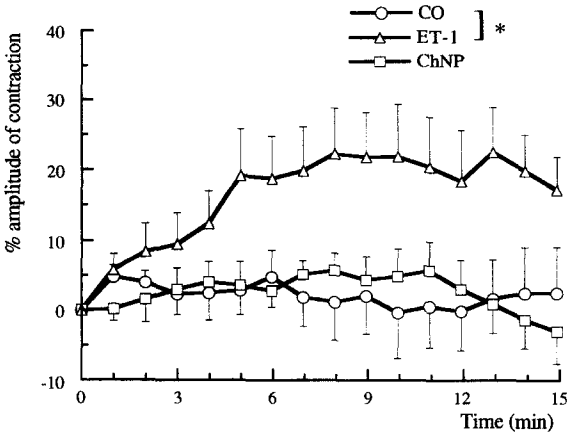

Fig. 1. Time course of the contractile responses of chick embryo ventricular cells to endothelin-1 (ET-1; △; 10^{-8} M), chick natriuretic peptide (ChNP; □; 3×10^{-7} M), and control perfusate (CO; ○). Results are expressed as a percentage of the amplitude of basal contraction, and represent the means ± S.E.M. of 10–12 experiments. Endothelin-1 increased significantly the mean amplitude of cell contraction (* $P < 0.05$), whereas chick natriuretic peptide had no effect.

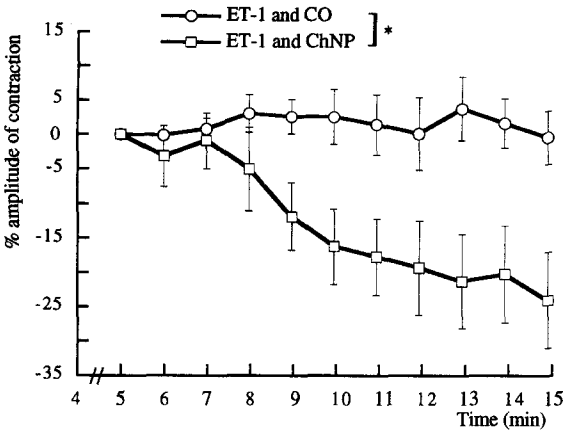

Fig. 2. Time course of the effects of chick natriuretic peptide (ChNP; □; 3×10^{-7} M) and control (CO; ○) perfusate on the contractility of chick embryo ventricular cells previously exposed to endothelin-1 (ET-1; 10^{-8} M) for 5 min. Results are expressed as a percentage of the amplitude of contraction at the 5th min of endothelin-1 perfusion, for comparison of control and ChNP and represent the means ± S.E.M. of 10 experiments. Endothelin-1 perfusion was constant throughout the experiment. The endothelin-1-induced increase in contractility was significantly reversed when chick natriuretic peptide was added to the endothelin-1-containing solution ($^*P < 0.05$).

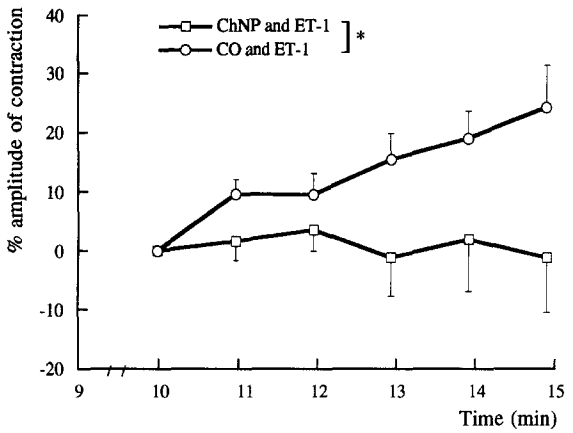

Fig. 3. Time course of the effects of endothelin-1 (ET-1; 10^{-8} M) on the contractility of chick embryo ventricular cells previously exposed to chick natriuretic peptide (ChNP; □; 3×10^{-7} M) or control perfusate (CO; ○) for 10 min. Results are expressed as a percentage of the amplitude of contraction at the 10th min of perfusion by ChNP or control, for comparison and represent the means ± S.E.M. of 10–12 experiments. Control and ChNP perfusions were constant throughout the experiments. ChNP prevented the endothelin-1-induced increase in contractility ($^*P < 0.05$).

increase in myocyte contractility when it was added 5 min after the outset of endothelin-1 perfusion ($P < 0.05$; Fig. 2), while it prevented the endothelin-1-induced increase in contractility observed when endothelin-1 was added 10 min after the onset of ChNP perfusion ($P < 0.05$; Fig. 3).

3.2. Quantification of the ChNP mRNA response to endothelin-1

To determine whether endothelin-1 increased the expression of the natriuretic peptide specific for chicken cardiac myocytes, we used Northern blot hybridization with a ChNP cDNA probe. This probe detected two mRNA bands with apparent molecular weights of 0.8 and 1.3 kb (Fig. 4). Scanning of each autoradiogram showed that the ratio between the two signals was constant (data not shown). We thus used the sum of the two mRNA signals for statistical analysis. In each experiment the results were expressed as the percentage variation in the ChNP mRNA signals normalized to 18S RNA signals in endothelin-1-treated cells relative to controls (Fig. 5).

ChNP mRNA accumulated markedly in chick cardiocytes exposed to 10^{-8} M endothelin-1. This increase was statistically significant as early as after 30 min of stimula-

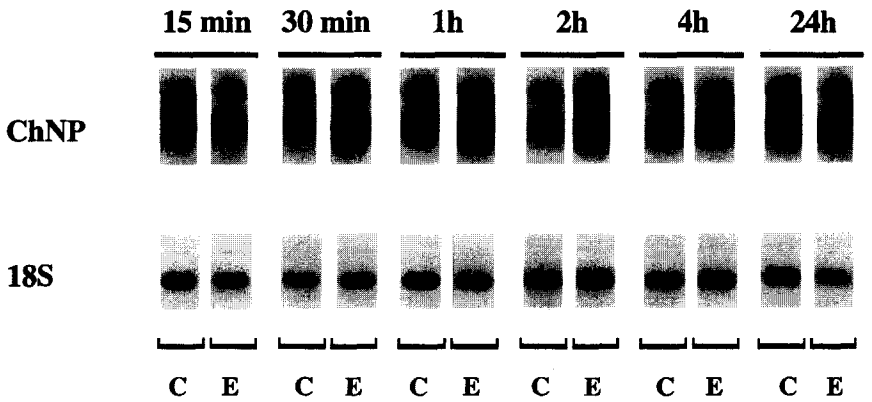

Fig. 4. Example of Northern blot analysis of chick natriuretic peptide mRNA extracted from cultured chick embryo ventricular cells stimulated by endothelin-1 (10^{-8} M) or control for different times (C: control; E: exposed to endothelin-1). Five micrograms of total RNA were loaded onto each lane. The ChNP cDNA probe detected two mRNA bands with apparent molecular weights of 1.3 and 0.8 Kb. Hybridization signals for 18S ribosomal RNA as an internal control are also shown. In response to endothelin-1, the ratio ChNP mRNA/18S rRNA increased after 30 min of stimulation, with a maximum effect at 2 h.

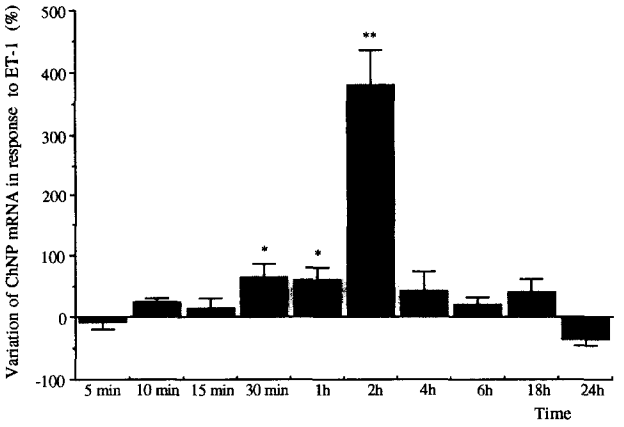

Fig. 5. Bar graph showing time-dependent stimulation of chick natriuretic peptide mRNA expression in response to endothelin-1 (ET-1; 10^{-8} M). Results are expressed as a percentage variation in the ChNP mRNA signals normalized to 18S RNA signals and represent the means ± S.E.M. of 6–12 experiments. Endothelin-1 increased the accumulation of ChNP mRNA as early as 30 min (* $P < 0.05$) after stimulation, with a maximal effect at the 2nd h (** $P < 0.01$), and without further effect after 4 h of stimulation.

tion with endothelin-1 treatment ($P < 0.05$). Maximal accumulation was observed after 2 h of stimulation (about a 5-fold increase compared to controls; $P < 0.01$) and no effect was observed thereafter.

4. Discussion

The main results of this study are as follows: (i) ChNP did not affect the contractility of chick cardiac myocytes in basal conditions but both prevented and antagonized the increase in myocyte contractility induced by endothelin-1; (ii) endothelin-1 transiently stimulated ChNP mRNA expression in these myocytes after as little as 30 min of exposure. These results suggest that ChNP expression in cardiac myocytes in response to endothelin-1 stimulation may be a negative feedback mechanism attenuating the endothelin-1-induced increase in myocyte contractility.

4.1. Effects of endothelin-1 and ChNP on contractility

Chick ventricular embryo cells in primary culture are completely free of hemodynamic and neurohumoral influences, and thus provide a system suitable for studying the direct contractile effects of peptides in vitro. Using the same model we have previously shown that endothelin-1 increases contractility to the same extent as the β-adrenoceptor agonist isoproterenol and the Ca^{2+}-channel agonist Bay k 8644 (Concas et al., 1989), with no change in cellular cAMP or cGMP concentrations (personal unpublished data), whereas rat ANP induces a slight decrease in myocyte contractility and a concentration-dependent (10^{-10}–10^{-6} M) increase in myocyte cGMP concentrations (Vaxelaire et al., 1989).

In the present work, we tested the effects of single concentrations of ChNP (3×10^{-7} M) and endothelin-1 (10^{-8} M) on chick cardiomyocyte contractility based on the information on (a) the concentration-effect curve of each component on cardiac contractility, and (b) the concentration-dependence of the interaction in other noncardiac tissue. The concentration of endothelin-1 (10^{-8} M) was selected from the concentration-effect curve (10^{-12}–10^{-7} M) determined in a previous work (Concas et al., 1989) as the maximum inotropic concentration on chick cardiomyocytes. In addition, this concentration was commonly used by others in cardiac (Gardner et al., 1991; Horio et al., 1993) and vascular tissues (Lang and Lewis, 1991). The concentration of ChNP (3×10^{-7} M) was extrapolated from the concentration-effect curves (10^{-10}–10^{-6} M) on cardiomyocyte contractility and intracellular cGMP content, established with rat ANP (Vaxelaire et al., 1989). The threshold concentrations for the decrease in contractility and the increase in cGMP were 10^{-8} M and 10^{-7} M, respectively, with a slight decrease in contractility at 2.5×10^{-7} M.

Endothelin-1 has been widely reported to increase cardiac contractility in vivo and in vitro (Ishikawa et al., 1988; Concas et al., 1989; Rubanyi and Polokoff, 1994). Although the subcellular mechanisms underlying this increase are not yet fully clear, it is generally accepted that endothelin-1 accelerates phosphatidylinositol turnover, resulting in increased production of inositol 1,4,5-trisphosphate and diacylglycerol; this increase has been shown to be involved in modulating intracellular Ca^{2+} mobilization as well as the sensitivity of contractile proteins to Ca^{2+} in various cell types (Takanashi and Endoh, 1991). This sensitization of cardiac filaments to intracellular Ca^{2+} could also be due to a rise in pH_i by stimulation of the sarcolemmal Na^{+}-H^{+} exchanger (Krämer et al., 1991).

The inotropic effect of natriuretic peptides has been less studied than that of endothelin-1, and the effect of ANP on cardiac contractility remains controversial. Different authors have reported an increase (Shapiro et al., 1986), no change (Kleinert et al., 1986), or a decrease (Seymour et al., 1985; Rankin and Swift, 1990) in cardiac contractility in vivo. Chicken natriuretic peptide (ChNP) is more closely related to BNP than to ANP (Miyata et al., 1988; Akisuki et al., 1991; Mesnard et al., 1993). Mesnard et al. (1993) have shown that a type A natriuretic peptide is not significantly expressed at any developmental stage of the chick heart, which mainly expresses a peptide very similar to type B natriuretic peptide. As the biological characterization of BNP is less advanced than that of ANP, we used a recent commercial ChNP preparation to study its effects on chick heart cells. The small difference between the slight decrease in contractility induced by rat ANP in our previous experiments (Vaxelaire et al., 1989) and the lack of effect of ChNP on contractility in this study may be due to a rightward shift of the concentration-effect curve of ChNP. This may be related to the different structures of the two

peptides. Indeed, the C-terminal Arg-Tyr sequence of mammalian ANP, thought to be necessary for peptide activity in rats, is replaced by Lys-Asn in ChNP (Miyata et al., 1988). The subcellular mechanisms underlying the change in cardiomyocyte contractility induced by ANP have rarely been studied. Three distinct natriuretic peptide receptors have been identified in mammal, and two of them, natriuretic peptide receptors A and B, activate guanylate cyclase, leading to intracellular accumulation of cyclic GMP (cGMP) (Wildey et al., 1991). cGMP may also participate in the negative inotropic effect of natriuretic peptides. Tei et al. (1990) observed a decrease in intracellular Ca^{2+} concentrations in response to human ANP in guinea pig atrial and ventricular myocytes, presumably occurring through cGMP-mediated activation of the sarcolemmal Ca^{2+} pump. In mammalian heart, cGMP has been shown to shorten the action potential duration by inhibiting the Ca^{2+} current, suggesting that the negative inotropic effects of cGMP are mediated by an inhibition of L-type Ca^{2+} channels (Lohmann et al., 1991).

The fact that ChNP prevented and reversed the inotropic effect of endothelin-1 points to a functional antagonism between the two peptides. By analogy with the relaxing effect of ANP on vascular smooth muscle contraction induced by norepinephrine, angiotensin II and K^+, these results, together with the lack of effect of ChNP on basal contractility, are reminiscent of the need for contractile prestimulation in the biological action of natriuretic peptides (Wildey et al., 1991). The most likely mechanism underlying the endothelin-1-ChNP interaction on cardiac contractility is the inhibition of phosphatidylinositol hydrolysis and protein kinase C activation by cGMP, an interaction which has been demonstrated in vascular smooth muscle cells (Rapoport, 1986; Hirata et al., 1990; Lang and Lewiss, 1991). In the present work, the inhibitory action of ChNP may be selective to the phosphatidylinositol hydrolysis-related positive inotropic effect. Indeed, we previously showed (Vaxelaire et al., 1989) that rat ANP antagonized the positive inotropic effect of angiotensin II, but not that of isoproterenol and the Ca^{2+} channel agonist Bay k 8644. In addition, ChNP was able to antagonize not only the increase in contractility induced by endothelin-1, but also that of angiotensin II (3×10^{-7} M), whereas it was devoid of effect on the isoproterenol 10^{-6} M-induced increase in contractility (unpublished data).

Whether the endothelin-1 and ChNP concentrations, used in the present study, are physiologically relevant and may be achieved in situ is difficult to determine because there are, to our knowledge, no available data. Although plasmatic concentration of endothelin-1 has been reported to be in the range of 0.2–2.0 10^{-12} M in humans and rats (Saito et al., 1989; Suzuki et al., 1989), interstitial concentrations are likely to be higher (Brunner, 1995), and endothelin-1, which is released by the cell and acts in an autocrine-paracrine fashion may be even more concentrated at the vicinity of its receptors. The same argument may be applied also to ChNP. The plasmatic levels of rat ANP (Goetz, 1988) and ChNP (Gray et al., 1991) are similar and in the range of 60–150 pg/ml, i.e. 1.7–5.0 10^{-11} M. Interestingly, the plasmatic ChNP concentrations are 9–25-fold higher than those of endothelin-1. Thus, according to these data and previous works from our group (Concas et al., 1989; Vaxelaire et al., 1989) and others (Rapoport, 1986; Lang and Lewiss, 1991) we selected a ChNP/endothelin-1 concentration ratio of 30.

4.2. Effects of endothelin-1 on ChNP mRNA expression

Endothelin-1 increased steady-state ChNP mRNA levels as early as 30 min after stimulation, with a maximum effect at the 2nd h of stimulation (about a 5-fold increase); no further effect was observed 4 h after stimulation. Such an early augmentation of natriuretic peptide mRNA expression by endothelin-1 has not previously been reported. Increased ANP mRNA expression in response to endothelin-1 has consistently been shown to occur 24 h after stimulation in neonatal rat ventricular cells (Gardner et al., 1991), and 8 h after stimulation in neonatal rat atrial cells (Suzuki et al., 1992); in the latter study BNP mRNA levels increased significantly after 16 h of stimulation by endothelin-1. Age and species differences may explain the discrepancy between these data and ours. As Mesnard et al. (1993) had reported that ANP is not expressed in chick heart, our results suggest that ChNP mRNA expression may not follow the same pattern as ANP mRNA expression. A 1-h atrial stretch induced a rapid increase in right-atrial BNP mRNA levels in perfused rat heart (Mantymaa et al., 1993), whereas no change in ANP mRNA levels was observed. In addition, the mRNA encoding ChNP, like other BNP, contains a specific sequence in the 3′ untranslated region containing ATTA repeats, which are associated with mRNA instability (Shaw and Kamen, 1986). This sequence is absent from ANP mRNA and may explain the transient expression of ChNP mRNA in response to endothelin-1, namely the decrease in ChNP mRNA concentration after 4 h of stimulation by endothelin-1. We did not evaluate ChNP secretion in response to endothelin-1, as endothelin-1-induced secretion of natriuretic peptides by cardiac tissue has been extensively reported (Hu et al., 1988; Sei and Glembotski, 1990; Gardner et al., 1991; Suzuki et al., 1992; Horio et al., 1992, 1993). The earliest secretion of ANP described to date occurred after 10 min of endothelin-1 stimulation (Sei and Glembotski, 1990). Also the time course of ChNP secretion following endothelin-1 stimulation remains to be established. It is likely that the observed rise in ChNP mRNA in response to ET-1 occurs subsequently, and, by increasing ChNP synthesis, compensates for the intracellular ChNP depletion.

The physiological significance of the endothelin-1-ChNP antagonism is unclear. However, the coronary microcirculation is close to cardiac muscle, at a distance

compatible, in terms of diffusion, with autocrine-paracrine regulation of cardiac contractility by vasoactive peptides produced by the adjacent endothelium. Brutsaert et al. (1988) have clearly established that myocardial contraction is modulated by endocardial endothelium, probably through the release of nitric oxide and an unidentified contracting factor, which respectively abbreviate and prolong muscle twitch contraction (Brutsaert et al., 1988; Shah and Lewis, 1993). Coronary vascular endothelium also releases nitric oxide and, probably, several other inotropic agents (Shah and Lewis, 1993), of which endothelin-1 is a good candidate. Our data also suggest that endothelin-1, by stimulating cardiocyte ChNP expression and secretion, could attenuate its own inotropic effect through a ChNP-mediated decrease in contractility. Endothelin-1 could also decrease its own secretion by endothelial cells in response to ChNP production, as suggested by the inhibition of the endothelin-1 secretory response to angiotensin II in porcine aorta by ANP and BNP (Kohno et al., 1992).

4.3. Conclusions

In conclusion, this study provides additional evidence for interactions between endothelin-1 and natriuretic peptides as autocrine/paracrine factors regulating cardiocyte contractility. Although ChNP had no effect on basal chick cardiocyte contractility, it both prevented and reversed the increase in contractility induced by endothelin-1. The early accumulation of ChNP mRNA in chick cardiocytes in response to endothelin-1 suggests that ChNP produced by cardiocytes in vivo can modulate the endothelin-1-induced increase in cardiocyte contractility through an autocrine mechanism.

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