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Positive inotropy mediated via CGRP receptors in isolated human myocardial trabeculae

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Abstract

Isometric contractile force were studied on isolated human myocardial trabeculae that were paced at 1.0 Hz in tissue baths. Alpha calcitonin gene-related peptide (α-CGRP) had a potent positive inotropic effect in most trabeculae from both the right atrium and left ventricle, and this effect was partially antagonized by the $CGRP_1$ receptor antagonist α-CGRP-(8-37) (10^{-6} M). Amylin and the $CGRP_2$ receptor agonist [Cys(acetylmethoxy)^{2,7}]CGRP had a positive inotropic effect in some trabeculae, whereas adrenomedullin had no inotropic effect. Using reverse transcriptase–polymerase chain reaction (PCR) mRNAs encoding the human calcitonin receptor-like receptor and the receptor associated modifying proteins (RAMPs) RAMP1, RAMP2, and RAMP3 were detected in human myocardial trabeculae from both the right atrium and left ventricle. In conclusion, functional $CGRP_1$ and $CGRP_2$ receptors may mediate a positive inotropic effect at both the atrial and ventricular level of the human heart. mRNAs for calcitonin receptor-like receptor and specific RAMPs further support the presence of CGRP receptors. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Amylin; Adrenomedullin; Calcitonin gene-related peptide (CGRP); Calcitonin receptor-like receptor; Myocardial contraction; Receptor associated modifying protein (RAMP)

1. Introduction

Calcitonin gene-related peptide (CGRP), amylin, and adrenomedullin are structurally related peptides with vasorelaxant effects (Muff et al., 1995). CGRP administered intravenously has beneficial hemodynamic effects in heart failure patients (Gennari et al., 1990), and the same has been demonstrated for adrenomedullin in an ovine model of heart failure (Rademaker et al., 1997). It is not clear, however, as to what extent the improvements in cardiac performance are due to indirect effects caused by vascular relaxation or a direct positive inotropic effect. A direct positive inotropic effect of CGRP has been demonstrated in vitro in the isolated auricle and trabeculae of the human right atrium (Du et al., 1994; Franco-Cereceda et al., 1987a), but to our knowledge not in human ventricular tissue. Regarding inotropic effects of amylin and adrenomedullin, there is sparse information, but positive inotropic effects have been reported for amylin in isolated guinea-pig atria (Giuliani et al., 1992) and in isolated porcine myocardial trabeculae (Saetrum Opgaard et al., 1999) and for adrenomedullin in isolated rat hearts (Szokodi et al., 1998).

Based on functional studies, two receptor subtypes for CGRP have been described, the $CGRP_1$ and the $CGRP_2$ receptors (Wimalawansa, 1996). The first efforts to clone a CGRP receptor resulted in receptors showing homology to the calcitonin receptor, and they were named calcitonin receptor-like receptors (Kapas and Clark, 1995; Njuki et al., 1993). A human receptor with the pharmacological profile of the $CGRP_1$ receptor was cloned in 1996, and its cDNA showed a 100% homology to the cDNA of the coding region of a human calcitonin receptor-like receptor (Aiyar et al., 1996). It is now widely accepted that this calcitonin receptor-like receptor is in fact the $CGRP_1$ receptor (Sams and Jansen-Olesen, 1998). A molecular coun-

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terpart to the functionally characterized $CGRP_2$ receptor has not yet been cloned.

Several studies have suggested that amylin and adrenomedullin may act via CGRP-like receptors in various tissues (Han et al., 1997; Poyner, 1997; Westfall and Curfman-Falvey, 1995). Recently it has been shown that CGRP and adrenomedullin bind to the same calcitonin receptor-like receptor; receptor specificity is however conferred by receptor associated modifying proteins (RAMPs), which are required to transport calcitonin receptor-like receptor to the plasma membrane (Buhlmann et al., 1999; McLatchie et al., 1998). Three different types of human RAMPs have been cloned, RAMP1, RAMP2, and RAMP3 (McLatchie et al., 1998). Calcitonin receptor-like receptor can function as either a CGRP receptor or an adrenomedullin receptor, depending on the type of RAMP associated with it. RAMP1 presents the calcitonin receptor-like receptor at the cell surface as a terminally glycosylated, mature glycoprotein with the specificity of a CGRP receptor. RAMP2 and probably also RAMP3 present calcitonin receptor-like receptor as a core glycosylated adrenomedullin receptor (Fraser et al., 1999; McLatchie et al., 1998). Attempts to clone an amylin receptor have resulted in the isolation of a cDNA encoding the calcitonin receptor (Chen et al., 1997). Furthermore, recent data show that RAMP1 and RAMP3 can generate amylin receptor phenotypes from a calcitonin receptor (Christopoulos et al., 1999; Muff et al., 1999).

The aim of the present study was to compare the effects of human α-CGRP, amylin and adrenomedullin on contractile force of isolated myocardial trabeculae from human right atria and left ventricles. Both functional and molecular approaches were used to identify CGRP-related receptors in human myocardial trabeculae. To assess the functional role of the $CGRP_1$ receptor, we used the selective $CGRP_1$ receptor antagonist, human α-CGRP-(8-37), which lacks seven terminal amino acid residues as compared to CGRP (Chiba et al., 1989). To study effects mediated by the $CGRP_2$ receptor, we used the selective $CGRP_2$ receptor agonist, [Cys(acetylmethoxy)^{2,7}]CGRP, which is a linear analog of CGRP (Dennis et al., 1989). Furthermore, from the presumption that calcitonin receptor-like receptor activated by RAMP1 represents a $CGRP_1$ receptor and calcitonin receptor-like receptor activated by RAMP2 or RAMP3 represents an adrenomedullin receptor, we wanted to determine the presence of mRNAs for calcitonin receptor-like receptor, RAMP1, RAMP2, and RAMP3 in myocardial tissue from human right atria and left ventricles.

2. Materials and methods

The investigation conforms with the principles outlined in the Declaration of Helsinki (Cardiovasc. Res. 1997; 35: 2–3). The collection of human tissues was in accordance with institutional guidelines, and the local ethics committee at each institution approved the project.

2.1. Functional experiments measuring myocardial contractions

2.1.1. General preparations

Myocardial trabeculae were excised from the inner surface of the right atrium and left ventricle of human hearts. The Rotterdam Heart Valve Bank (Bio Implant Services Foundation/Eurotransplant Foundation) kindly provided the hearts after removal of the aortic and pulmonary valves for homograft valve implantation. The hearts came from 11 males and 7 females (age: 15–63 years with a mean ± S.E.M. of 47 ± 2.5 years). They were all previously healthy individuals that had died from cerebrovascular accidents or head trauma. The hearts were initially stored in a chilled, sterile organ protection solution (UW, Eurocollins; or HTK-Brettschneider) (Ploeg et al., 1992) and prior to experiments, were placed in chilled Krebs buffer of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, $KHPO_4$ 1.2, and glucose 8.3. Only trabeculae that were free from the wall of the heart and with a diameter less than 1 mm were used. Care was taken not to damage the endothelial surface of the tissue. The trabeculae were mounted in organ baths (15 ml) containing the above described Krebs buffer, which was kept at 37°C and continuously gassed with a mixture of 95% O_2 and 5% CO_2, giving a pH of approximately 7.4. The ends of the trabeculae were tied with silk sutures and connected to a Harvard transducer for measurement of isometric tension. The trabeculae were paced at 1.0 Hz using field stimulation (5 ms, voltage 20% above threshold for initiation of contractile response), through electrodes placed in the organ baths. Resting tension was set to approximately 750 mg for atrial trabeculae and 1950 mg for ventricular trabeculae (Du et al., 1994). During continuous pacing, the tissues were allowed to stabilize for approximately 90 min before the baseline contractile amplitude was measured.

2.1.2. Concentration–response curves

Concentration–response curves for noradrenaline were obtained in some of the trabeculae, showing that a concentration of 10^{-5} M gave a nearly maximum response to noradrenaline. This concentration of noradrenaline was used to test the responsiveness of all trabeculae and for comparison with other positive inotropic agents. Trabeculae with an increase in contractile force of less than 25 mg upon exposure to 10^{-5} M noradrenaline were excluded from the study.

After several wash outs with normal Krebs-buffer and stabilization at baseline contractile force, cumulative concentrations of peptide agonists were added and changes in contractile force were measured. At the end of the experiments, the reactivity of the trabeculae was again tested by exposure to noradrenaline (10^{-5} M).

2.1.3. Analysis of data

Maximum effect obtained with an agonist (E_{max}) and the negative logarithm of the concentration of agonist that elicited half maximum effect (pEC_{50}) were derived from concentration–response curves on each trabecula. Values are given as mean ± S.E.M. For multiple comparisons of responses to different agonists, we used one factor analysis of variance (ANOVA) followed by Fisher's Protected Least Significant Difference test. When comparing responses to agonist with or without antagonist, we used Mann–Whitney *U*-test to determine statistical significance with respect to differences in E_{max} and pEC_{50} values. Statistical significance was assumed when $P < 0.05$.

2.2. Molecular biology experiments

2.2.1. Isolation of total RNA

Myocardial trabeculae were obtained from the right atrium and left ventricle of human explanted hearts in connection with heart transplantation, frozen in liquid nitrogen and stored at −80°C. Total cellular RNA was isolated by the method of acid guanidinium thiocyanate/phenol/chloroform extraction (Chomczynski and Sacchi, 1987). Frozen tissue was homogenized in 1.5 ml Eppendorf tubes using 0.5 ml of denaturing solution containing 4 M guanidinium isothiocyanate, 25 mM sodium citrate (pH 7.0), 0.5% *N*-lauroylsarcosine, and 0.1 M 2-β-mercaptoethanol. In order, homogenates were mixed thoroughly with 50 μl 2 M sodium acetate (pH 4.0), 0.5 ml water saturated phenol, and finally 100 μl chloroform/isoamyl alchohol (49:1 v/v). The mixture was vortexed for 15 s and kept on ice for 15–30 min. After centrifugation at 13000 × *g* for 15 min at 4°C, the upper aqueous phase was transferred to a new disposable tube and RNA was precipitated twice with isopropanol at −20°C. The RNA pellet was finally washed with 70% ethanol, dried, dissolved in 20 μl of diethylpyrocarbonate-treated water, and stored at −20°C until use. The amount and purity of RNA was evaluated by absorption at 260 nm, using a DU-64 spectrophotometer (Beckman Instruments, Sweden), and the ratio of absorption (260:280 nm) of all preparations was between 1.6 and 1.8. Finally, all samples were subjected to gel electrophoresis to confirm the integrity of the 18 and 28S ribosomal RNAs.

2.2.2. Oligonucleotide design

The primers for calcitonin receptor-like receptor and RAMPs mRNA, used for reverse transcriptase–polymerase chain reaction (PCR) assays, were as published by Sams and Jansen-Olesen (1998):

calcitonin receptor-like receptor forward: 5′TGCTCTGTGAAGGCATTTAC3′,
calcitonin receptor-like receptor reverse: 5′CAGAATTGCTTGAACCTCTC3′ (497 bases),
RAMP1 forward: 5′GAGACGCTGTGGTGTGACTG3′,
RAMP1 reverse: 5′TCGGCTACTCTGGACTCCTG3′ (445 bases),
RAMP2 forward: 5′GGACGGTGAAGAACTATGAG3′,
RAMP2 reverse: 5′ATCATGGCCAGGAGTACATC3′ (283 bases),
RAMP3 forward: 5′TGGAAGTGGTGCAACCTGTC3′,
RAMP3 reverse: 5′CACGGTGCAGTTGGAGAAGA3′ (159 bases).

2.2.3. Reverse transcriptase–PCR

The reverse transcription of total RNA to cDNA and subsequent PCR was carried out using the GeneAmp RNA PCR kit (Perkin Elmer, Denmark) in a RoboCycler Gradient 40 (Stratagene). First strand cDNA was synthesized from 1 μg total RNA in a 20 μl reaction volume following the standard reverse transcription protocol (GeneAmp RNA PCR kit) and using random hexamers as primers. The reaction was incubated at 42°C for 15 min, heated to 99°C for 5 min, and chilled to 5°C for 5 min. For each mRNA extract, a reverse trancriptase negative control was performed by substituting the reverse transcriptase enzyme with nuclease free water in the reaction mixture. The resultant cDNA was amplified by PCR in a final volume of 25 μl, following the standard PCR protocol (GeneAmp RNA PCR kit), and Platinum™ Taq DNA Polymerase (GibcoBRL) was used as the thermostable enzyme. The PCR reaction was carried out by using four linked files; file 1: initial denaturation step 5 min at 95°C for 1 cycle, file 2: denaturation 60 s at 95°C, annealing 90 s at 63°C and elongation 30 s at 72°C for 40 cycles, file 3: elongation 7 min at 72°C for 1 cycle.

2.2.4. Electrophoretic analysis of PCR products

After PCR, a 10-μl aliquot from each PCR product was electrophoresed on a 2% agarose gel, containing 0.5 μg/ml ethidium bromide, in TBE buffer (89 mM Tris–borate, 2 mM EDTA, pH 8.0) at 5 V/cm for 1 h. The DNA Ladder 100 bp (GibcoBRL) was used as the molecular weight marker.

2.3. Drugs

The following drugs were purchased from the sources indicated: noradrenaline (Sigma, St. Louis, MO, USA); human amylin, human adrenomedullin, human α-CGRP and human α-CGRP-(8-37) (Bachem, Bubendorf, Switzerland); and diacetoamidomethyl cysteine CGRP [Cys(acetylmethoxy)^{2,7}]CGRP] (Peninsula, St. Helens, UK). The drugs were dissolved in distilled water. All

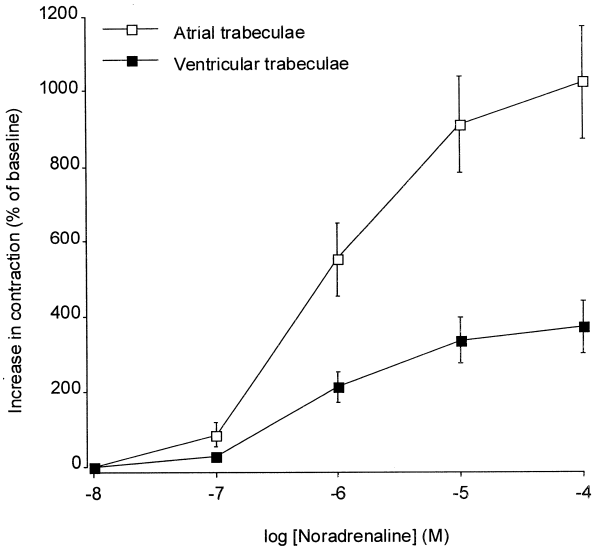

Fig. 1. Positive inotropic effect of noradrenaline. Concentration–response curves for noradrenaline on human trabeculae from right atria ($n = 21$) and left ventricles ($n = 20$). Each point shows the mean increase in contractile amplitude measured as percentage of baseline contractile amplitude for each individual trabecula. The S.E.M. are shown as vertical bars. The E_{max} values were significantly higher in atrial compared to ventricular trabeculae ($P < 0.001$).

reagents for the molecular biology experiments were purchased from Sigma.

3. Results

3.1. Functional experiments

3.1.1. Noradrenaline

Concentration–response curves for noradrenaline up to a concentration of 10^{-4} M were performed on some of the trabeculae ($n = 21$ atrial and 20 ventricular trabeculae). The baseline contractile force (mean ± S.E.M.) was 45 ± 6 mg in the atrial and 230 ± 54 mg in the ventricular trabeculae. The E_{max} values for noradrenaline were 926 ± 130% in atrial and 342 ± 61% in ventricular trabeculae; these values represent mean ± S.E.M. of the increase in contractile force measured as percentage of baseline contractile force in each individual trabecula (Fig. 1). The E_{max} values for noradrenaline were significantly ($P < 0.001$) higher in atrial compared to ventricular tissue.

As can be seen from Fig. 1, the response to noradrenaline seemed to be essentially at a maximum at a concentration of 10^{-5} M, and this concentration of noradrenaline was used to assess the responsiveness of all trabeculae prior to testing different agonists.

3.1.2. α-CGRP

α-CGRP, tested in cumulative concentrations from 10^{-11} to 3×10^{-7} M, increased contractile force in 8 out

Table 1
Inotropic effects in atrial and ventricular trabeculae
Positive inotropic effect of α-CGRP without and after preincubation with α-CGRP-(8-37) (10^{-6} M), and the effect of [Cys(acetylmethoxy)^{2,7}]CGRP (CysACM-CGRP), amylin and adrenomedullin (ADM) tested on isolated human trabeculae from right atria and left ventricles. All agonists were tested at baseline contractile force. Due to the lack of positive inotropic effect of adrenomedullin when tested at baseline (= ''ADM-baseline''), the peptide was also tested after stimulating the trabeculae with noradrenaline (10^{-5} M) (= ''ADM-with NA'') in order to better assess a possible negative inotropic effect. ''n_1'' = number of trabeculae tested, ''n_2'' = number of trabeculae responding to agonist. ''Baseline (mg)'' = contractile amplitude before exposure to agonist, measured in mg. ''Increase NA (%)'' = increase in contractile amplitude after exposure to noradrenaline (10^{-5} M), measured as percentage of baseline contractile amplitude. ''$E_{max} - n_1$ (%)'' = maximum increase in contractile amplitude induced by agonist, measured as percentage of baseline contractile amplitude and calculated from all trabeculae tested. ''$E_{max} - n_2$ (%)'' = E_{max} value calculated as for ''$E_{max} - n_1$ (%)'' except that non-responding trabeculae are excluded. Calculations were done on each individual trabecula and values given as mean ± S.E.M.

Agonist	n_1	n_2	Baseline (mg)	Increase NA (%)	$E_{max} - n_1$ (%)	$E_{max} - n_2$ (%)	pEC_{50}
Atria							
CGRP	11	8	45 ± 16	952 ± 165	164 ± 56	226 ± 64	7.79 ± 0.11
α-CGRP-(8-37) + CGRP	11	6	40 ± 13	1347 ± 516	92 ± 41	169 ± 61	7.48 ± 0.19[a]
CysACM-CGRP	12	7	75 ± 19	1012 ± 355	48 ± 16	83 ± 19	
Amylin	9	2	43 ± 14	1255 ± 319	6 ± 4	27 ± 7	
ADM-baseline	6	0	63 ± 15	1055 ± 362	0	0	
ADM-with NA	10	0	41 ± 8	1090 ± 299	0	0	
Ventricles							
CGRP	13	11	199 ± 54	417 ± 81	64 ± 18[b]	76 ± 19[b]	7.77 ± 0.09
α-CGRP-(8-37) + CGRP	13	8	166 ± 33	273 ± 43	27 ± 9	44 ± 12	7.39 ± 0.12[a]
CysACM-CGRP	11	4	193 ± 48	352 ± 94	6 ± 4	16 ± 10	
Amylin	11	3	180 ± 46	393 ± 120	6 ± 3	21 ± 6	
ADM-baseline	9	0	251 ± 34	204 ± 46	0	0	
ADM-with NA	10	0	182 ± 30	352 ± 69	0	0	

[a] In both atria and ventricles, the pEC_{50} values for α-CGRP were significantly ($P < 0.05$) lower after preincubation with α-CGRP-(8-37) (10^{-6} M), whereas the E_{max} values were not significantly different with or without the antagonist, regardless if including the non-responding trabeculae into the calculation or not.

[b] The E_{max} values for α-CGRP were significantly ($P < 0.01$) lower in ventricular compared to atrial trabeculae, both when including and not including the non-responding trabeculae.

of 11 atrial trabeculae tested and in 11 out of 13 ventricular trabeculae tested. The mean E_{max} value for α-CGRP was significantly ($P < 0.01$) higher in atrial compared to ventricular trabeculae, regardless if calculated only from responding trabeculae or if also including the non-responding trabeculae ($E_{max} = 0$). The pEC_{50} values were similar in atrial and ventricular tissue (details in Table 1 and Fig. 2).

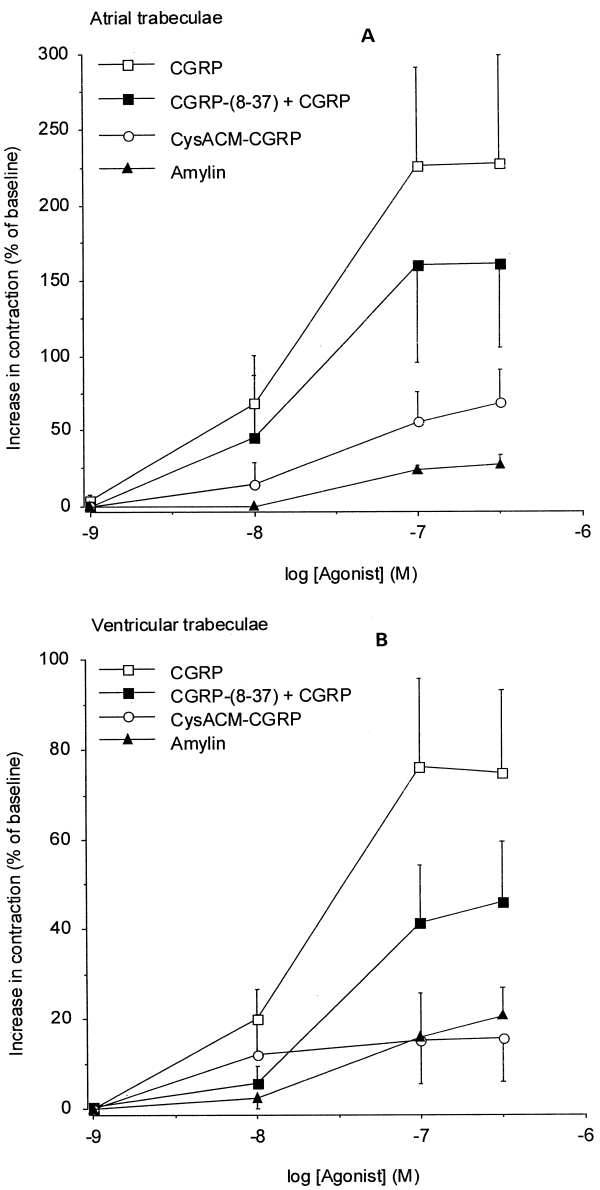

Fig. 2. Positive inotropic effects of CGRP and amylin. Comparison of positive inotropic effects of α-CGRP without and after preincubation with the $CGRP_1$ receptor antagonist α-CGRP-(8-37) (10^{-6} M), as well as the positive inotropic effects of the $CGRP_2$ receptor agonist [Cys(acetylmethoxy)^{2,7}]CGRP (CysACM-CGRP) and amylin, tested on isolated trabeculae from human right atria (A) and left ventricles (B). Adrenomedullin had no effect in either atrial or ventricular tissue. Each point shows the mean increase in contractile amplitude measured as percentage of baseline contractile amplitude for each individual trabecula. Only trabeculae responding to agonist are included. The S.E.M. are shown as vertical bars. Further information in Table 1.

In trabeculae incubated with the $CGRP_1$ receptor antagonist α-CGRP-(8-37) (10^{-6} M), the pEC_{50} values for α-CGRP were significantly ($P < 0.05$) decreased in both atrial and ventricular trabeculae. The E_{max} values for α-CGRP also tended to be lower after preincubation with the antagonist in both atrial and ventricular trabeculae; however, these differences were not significant, regardless if calculated from only responding trabeculae or if also including the non-responding trabeculae into the calculation (Table 1 and Fig. 2).

3.1.3. [Cys(acetylmethoxy)^{2,7}]CGRP

[Cys(acetylmethoxy)^{2,7}]CGRP, a selective $CGRP_2$ receptor agonist, was tested in the concentration range from 10^{-11} to 3×10^{-7} M. It caused an increase in contractile force in 7 out of 12 atrial and in 4 out of 11 ventricular trabeculae tested. The E_{max} values for [Cys(acetylmethoxy)^{2,7}]CGRP were significantly ($P < 0.05$) lower than those of α-CGRP in both atrial and ventricular trabeculae, with the limitation that in atrial trabeculae, the concentration–response curve for [Cys(acetylmethoxy)^{2,7}]-CGRP was still increasing at the highest concentration tested. For this reason and due to the relatively weak responses, no pEC_{50} values for [Cys(acetylmethoxy)^{2,7}]CGRP were calculated. When comparing responses to [Cys(acetylmethoxy)^{2,7}]CGRP in atrial to those in ventricular trabeculae, the E_{max} value tended to be higher in atrial trabeculae, although not statistically significant (Table 1 and Fig. 2).

3.1.4. Amylin

Amylin added in cumulative concentrations (10^{-11}–3×10^{-7} M) increased contractile force in 2 out of 9 atrial and in 3 out of 11 ventricular trabeculae tested (Table 1 and Fig. 2). Significance levels were not calculated due to the small number of responding trabeculae.

3.1.5. Adrenomedullin

Adrenomedullin tested in cumulative concentrations from 10^{-11} to 3×10^{-7} M did not influence the baseline contractile force of either atrial ($n = 6$) or ventricular ($n = 9$) trabeculae (Table 1). To better assess a possible negative inotropic effect, adrenomedullin was also tested in cumulative concentrations (10^{-11}–3×10^{-7} M) on trabeculae stimulated with noradrenaline in a concentration of 10^{-5} M. Even here, no changes in contractility were seen in either atrial ($n = 10$) or ventricular ($n = 10$) trabeculae (Table 1).

3.1.6. Responding versus non-responding trabeculae

Neither among atrial nor among ventricular trabeculae were there any significant differences in baseline contractile amplitude or noradrenaline-induced responses between those trabeculae responding to α-CGRP (with or without α-CGRP-(8-37)), [Cys(acetylmethoxy)^{2,7}]CGRP, and amylin compared to those not responding (data not shown).

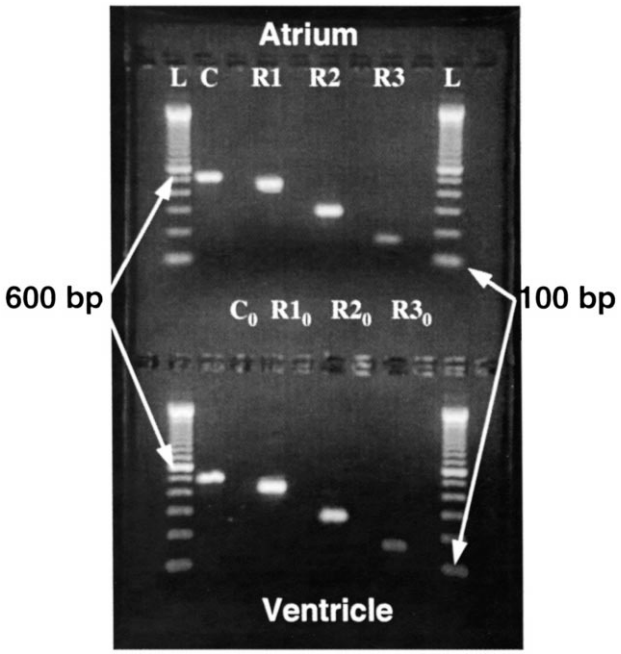

Fig. 3. mRNA for calcitonin receptor-like receptor and RAMPs. Demonstration of mRNA encoding calcitonin receptor-like receptor, RAMP1, RAMP2, and RAMP3, in the right atrium and left ventricle of the human heart by reverse transcriptase–PCR. L: 100 base pair ladder, C: calcitonin receptor-like receptor (497 bases), R1: RAMP1 (445 bases), R2: RAMP2 (283 bases), R3: RAMP3 (159 bases). C_0, $R1_0$, $R2_0$, $R3_0$: Negative controls without the reverse transcriptase enzyme. Bands with mRNA encoding calcitonin receptor-like receptor, RAMP1, RAMP2, and RAMP3, are shown in the respective lanes in the atrium and ventricle of the human heart. No bands are present in the negative control lanes.

3.2. Molecular biology experiments

Gel electrophoresis of the reverse transcriptase–PCR products produced bands of the expected sizes corresponding to mRNAs encoding calcitonin receptor-like receptor, RAMP1, RAMP2, and RAMP3, in the right atrium and left ventricle of the human heart (Fig. 3). No bands were seen in the negative controls without the reverse transcriptase enzyme.

4. Discussion

4.1. Noradrenaline

Exposure to noradrenaline caused an enormous increase in contractile force, more than ninefold in atrial and more than threefold in ventricular trabeculae. The responses to noradrenaline were significantly stronger in atrial compared to ventricular trabeculae when measured as percentage of baseline contractile amplitude. Noradrenaline is known to mediate its positive inotropic effect via β_1-adrenoceptors to evoke maximum positive inotropic effects in both atria and ventricles (Brodde et al., 1992). If the maximum responses to noradrenaline are taken as an approximation of maximum positive inotropic responses in the human tissues, then our results would suggest a higher potential for modulation of contractile force in atrial than in ventricular tissue. Whereas the contribution of atrial contraction to ventricular filling in healthy individuals at resting conditions has been estimated to only 29% and 23% for the left and right atrium, respectively (Jarvinen et al., 1994; Matsuda et al., 1983); this raises interesting questions as to the role of the atria during conditions with diminished cardiac reserve. Both exercise and pathophysiological conditions, like heart failure, are accompanied by an increase in sympathetic tone (Dimsdale and Moss, 1980; Porter et al., 1990), and atrial contraction seems to be particularly important to maintain cardiac output and exercise performance under critical conditions (Pardaens et al., 1997).

4.2. CGRP

CGRP, which appears in two isoforms, α- and β-CGRP (Amara et al., 1985), has been demonstrated both in association with the coronary vasculature and myocardial cells (Gulbenkian et al., 1993; Saetrum Opgaard et al., 1995; Wharton and Gulbenkian, 1989). CGRP is a potent vasorelaxant that causes fall in blood pressure, increased heart rate, and has a positive inotropic effect when administered intravenously in healthy volunteers (Franco-Cereceda et al., 1987b). It has not been clear if this positive inotropic effect represents a direct effect on the heart or if it is of reflex origin due to the concomitant fall in blood pressure. Most studies on isolated mammalian hearts and myocardial tissue could either not demonstrate any positive inotropic effect of CGRP or demonsrate effect only on the atria and not on the ventricles (Du et al., 1994; Franco-Cereceda et al., 1987a; Ishikawa et al., 1987; Raddino et al., 1997; Rigel et al., 1989; Sigrist et al., 1986). This led to the assumption that CGRP is physiologically important in the atria rather than in the ventricles and that the positive inotropic effect of CGRP seen in vivo is of reflex origin due to reduced blood pressure. Positive inotropic effects of CGRP have been previously demonstrated on isolated rat ventricular cardiomyocytes (Bell and McDermott, 1994), and we recently demonstrated a positive inotropic effect of CGRP in isolated trabeculae from porcine right atria and left ventricles (Saetrum Opgaard et al., 1999). The present study, to our knowledge, is the first to demonstrate a direct positive inotropic effect of CGRP in human ventricular tissue. That CGRP may exert a direct positive inotropic effect, at least during certain pathophysiological conditions, is supported by a previous in vivo study where CGRP given intravenously to patients with congestive heart failure improved myocardial contractility without any consistent change in arterial pressure or heart rate (Gennari et al., 1990). The significantly stronger positive inotropic effect of CGRP in atrial compared to

ventricular trabeculae in our study could point towards a more important role of CGRP in the atria than in the ventricles. Apart from the previously demonstrated differences in functional responses to CGRP between atria and ventricles, as discussed above, a previous study demonstrated a fourfold higher level of CGRP-like-immunoreactivity in the atria than in the ventricles of hearts from guinea pigs and humans (Franco-Cereceda et al., 1987c). Furthermore, an abundance of high affinity binding sites for CGRP have been demonstrated in the atria of laboratory animals, but relatively little in the ventricles (Rubino and Burnstock, 1996; Sigrist et al., 1986; Van Rossum et al., 1994).

4.3. CGRP receptors

The antagonistic effect of the $CGRP_1$ receptor antagonist α-CGRP-(8-37) in our study would suggest that $CGRP_1$ receptors mediate positive inotropic responses in human atrial and ventricular trabeculae, but with limited conclusions as only one concentration of the antagonist was used. Previous studies on other tissues have concluded that α-CGRP-(8-37) acts as a competitive antagonist at the $CGRP_1$ receptor (Mimeault et al., 1992; Poyner et al., 1992). The fact that α-CGRP-(8-37) in our study did not cause a strict rightward shift of the dose–response curves, along with the tendency towards lower E_{max} values with the antagonist (Fig. 2), may suggest that other receptors than the $CGRP_1$ receptor are also involved. The positive inotropic effect of the $CGRP_2$ receptor agonist [Cys(acetylmethoxy)^{2,7}]CGRP observed in some atrial and ventricular trabeculae would suggest that $CGRP_2$ receptors also mediate positive inotropic responses. However, due to the variability in responses between different trabeculae, conclusions ought to be avoided. Nevertheless, our findings find support from a previous study of isolated rat ventricular cardiomyocytes, where the positive inotropic effect of CGRP was potently antagonised by α-CGRP-(8-37), and where [Cys(acetylmethoxy)^{2,7}]CGRP also had a contractile effect, though less potent than CGRP (Bell and McDermott, 1994). In electrically driven isolated guinea pig left atria, however, [Cys(acetylmethoxy)^{2,7}]CGRP up to a concentration of more than 1 μM did not influence contractile force, whereas CGRP had a strong and potent positive inotropic effect (Dennis et al., 1989). Previous experiments on porcine myocardial trabeculae have suggested the involvement of both functional $CGRP_1$ and $CGRP_2$ receptors in the mediation of positive inotropic responses (Saetrum Opgaard et al., 1999), but this is the first study to suggest the functional involvement of $CGRP_1$ and $CGRP_2$ receptors in human atrial and ventricular trabeculae. The variations in responses in different studies and the fact that [Cys(acetylmethoxy)^{2,7}]CGRP and CGRP in the present study as well as in the previous study with porcine myocardial trabeculae had a positive inotropic effect in only some of the trabeculae, could have its explanation in different density or function/activation of $CGRP_1$ and $CGRP_2$ receptors or second messenger mechanisms. Furthermore, a previous competitive receptor binding study in rat atrium demonstrated that α-CGRP did bind with higher affinity to what appeared to be CGRP receptors than did [Cys(acetylmethoxy)^{2,7}]CGRP and amylin (Van Rossum et al., 1994).

Our present finding of mRNAs encoding calcitonin receptor-like receptor and RAMP1 further supports the presence of functional $CGRP_1$ receptors in human atrial and ventricular trabeculae according to the hypothesis recently proposed by McLatchie et al. (1998) and confirmed by other studies (Buhlmann et al., 1999; Fraser et al., 1999). Regarding the functionally classified $CGRP_2$ receptor, its molecular counterpart has not been cloned yet, and the mechanisms behind the function of this receptor are still unclear. It cannot be excluded that specific RAMPs are necessary for the activation of both receptor subtypes, which might aid in explaining why inotropic effects to CGRP and to the $CGRP_2$ receptor agonist [Cys(acetylmethoxy)^{2,7}]CGRP were seen in some trabeculae and not in others. It is further possible that multiple receptors and/or mechanisms may be activated by CGRP and related peptides, and in a previous study on isolated rabbit cardiac ventricular myocytes, it was actually shown that CGRP as well as adrenomedullin had a negative inotropic effect (Ikenouchi et al., 1997). From our study on human tissue, it can however be concluded that CGRP has the potential to increase cardiac contractility, not only indirectly through its vasorelaxant effect, but also through a direct positive inotropic effect on the heart at both the atrial and the ventricular level. Together with the previous demonstration of elevated plasma-levels of CGRP in congestive heart failure (Ferrari et al., 1991) and the demonstration in vivo of a presumably direct positive inotropic effect of CGRP in heart failure patients (Gennari et al., 1990), this suggests that CGRP may play an important role as a direct inotropic agent during heart failure.

4.4. Amylin

Amylin has considerable homology with CGRP (Cooper et al., 1987; Westermark et al., 1986) and is present, among other tissues, also in sensory ganglia (Ferrier et al., 1989). Amylin has a vasorelaxant effect in various vascular beds (Muff et al., 1995; Westfall and Curfman-Falvey, 1995), but the knowledge about the effect of amylin on myocardial contractility is very limited. In our study, amylin had a positive inotropic effect in only some atrial and ventricular trabeculae, and from our studies we can not conclude as to which receptors are involved. The demonstration of mRNA for RAMP1 and RAMP3 in atrial and ventricular trabeculae could however have implications for the recently proposed hypothesis that these RAMPs combine with a calcitonin receptor to generate amylin receptors (Christopoulos et al., 1999; Muff et al., 1999). A

previous study on isolated guinea-pig left atrium concluded that amylin has a positive inotropic effect mediated via $CGRP_2$ receptors, but with lower potency than that of CGRP (Giuliani et al., 1992). From a study on rat ventricular cardiomyocytes, it was concluded that amylin has a positive inotropic effect mediated via $CGRP_1$ receptors, but also less potent than that of CGRP (Bell and McDermott, 1995). A weak positive inotropic effect of amylin has also been demonstrated in porcine atrial trabeculae (Saetrum Opgaard et al., 1999). In that study, as well as in the present study on human tissue, only some trabeculae responded to amylin. The fact that baseline contractile amplitude and responses to noradrenaline in the present study were not significantly different in the groups that are responding compared to the groups that are not responding to the peptides, makes it unlikely that absence of peptide-induced responses were due to tissue-damage. The variation in responses might however reflect different activation of specific receptors, and could fit into the concept that specific RAMPs are necessary for the activation of these receptors. It has further been shown that the cellular phenotype of the calcitonin family of receptors is likely to be dynamic in regard to the level and combination of both the receptor and the RAMP proteins (Christopoulos et al., 1999).

4.5. Adrenomedullin

Adrenomedullin, which has structural similarities to CGRP and amylin (Kitamura et al., 1993), is abundantly present in the mammalian heart (Jougasaki et al., 1995a; Sakata et al., 1994). Both plasma levels and myocardial levels of adrenomedullin are increased in heart failure patients (Jougasaki et al., 1995b). The failing human heart actually secretes adrenomedullin (Jougasaki et al., 1996; Nishikimi et al., 1997), suggesting that this peptide may function as a paracrine and/or autocrine factor as well as a circulating hormone. In the present study, we could not detect any inotropic effects of adrenomedullin, neither positive nor negative, on atrial or ventricular trabeculae, consistent with previous experiments on porcine myocardial trabeculae (Saetrum Opgaard et al., 1999). It is therefore not likely that adrenomedullin functions as direct regulator of contractile force in the porcine and human heart under normal conditions. However, a role of adrenomedullin in the cardiac regulation is suggested by the presence of specific binding sites for adrenomedullin in rat hearts, where adrenomedullin, amylin, and CGRP, all competed for [^{125}I]adrenomedullin binding (Owji et al., 1995). The present demonstration of mRNAs encoding calcitonin receptor-like receptor, RAMP2 and RAMP3 in human atrial and ventricular myocardium, also suggests the presence of specific adrenomedullin receptors, presuming that calcitonin receptor-like receptor together with RAMP2 or RAMP3 confers receptor specificity to adrenomedullin (Buhlmann et al., 1999; Fraser et al., 1999; McLatchie et al., 1998). A positive inotropic effect of adrenomedullin has previously been demonstrated in isolated rat hearts (Szokodi et al., 1996, 1998), whereas adrenomedullin had a negative inotropic effect on isolated rabbit ventricular myocytes (Ikenouchi et al., 1997). A previous study with intravenous administration of adrenomedullin in an ovine model of heart failure demonstrated beneficial hemodynamic and renal effects with increased cardiac output, which might however be due to reduced peripheral resistance (Rademaker et al., 1997). Furthermore, intravenous infusion of adrenomedullin in conscious sheep caused an increase in heart rate and cardiac output which was concomitant with a lowering of mean arterial blood pressure, but it was concluded that adrenomedullin also had a direct positive inotropic effect (Parkes and May, 1997). Another study showed that pressure overload acutely stimulates ventricular adrenomedullin gene expression in conscious normotensive rats, suggesting a potential beneficial role for endogenous adrenomedullin production in the heart against cardiac overload (Romppanen et al., 1997). It is thus possible that adrenomedullin has a functional role in the regulation of cardiac contractility at least during certain pathophysiological conditions. The presence of specific receptors for adrenomedullin on the other hand does not necessarily imply that these receptors mediate inotropic effects.

4.6. Conclusion

α-CGRP had a potent positive inotropic effect in most of the atrial and ventricular trabeculae. As for noradrenaline, the E_{max} value for α-CGRP was significantly higher in atrial compared to ventricular trabeculae, suggesting a higher potential for modulation of contractile force in atrial than in ventricular tissue. Studies with the $CGRP_1$ receptor antagonist human α-CGRP-(8-37) and the $CGRP_2$ receptor agonist [Cys(acetylmethoxy)^{2,7}]CGRP may suggest that both $CGRP_1$ and $CGRP_2$ receptors mediate positive inotropic responses. The detection of mRNAs encoding calcitonin receptor-like receptor and RAMP1 in both atrial and ventricular trabeculae supports the presence of the $CGRP_1$ receptor, which according to present knowledge is identical to calcitonin receptor-like receptor activated by RAMP1, whereas the mechanism behind the functional $CGRP_2$ receptor in this context is still unknown. Amylin had a positive inotropic effect in only some atrial and ventricular trabeculae, and our detection of mRNAs for RAMP1 and RAMP3 in human atrial and ventricular trabeculae may have implications for the presence of functional amylin receptors according to a newly presented hypothesis. No direct inotropic effects of adrenomedullin, neither positive nor negative, were seen in this study. The detection of mRNAs for calcitonin receptor-like receptor, RAMP2 and RAMP3, does however suggest the presence of adrenomedullin receptors, but their possible role in the cardiac regulation remains unclear.

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