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Positive inotropy of calcitonin gene-related peptide and amylin on porcine isolated myocardium

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Abstract

Isolated porcine myocardial trabeculae from right atria and left ventricles were paced at 1.5 Hz in tissue baths, and changes in isometric contractile force upon exposure to agonist were studied. Alpha calcitonin gene-related peptide (α-CGRP) increased contractile force in nearly half of the trabeculae, whereas the selective $CGRP_2$ receptor agonist [Cys(acetylmethoxy)^{2,7}]-CGRP had effect in only a few. Preincubation with the $CGRP_1$ receptor antagonist α-CGRP-(8-37) (10^{-6} M) almost completely blocked positive inotropic responses to α-CGRP. Amylin had weak positive inotropic effects in some atrial, but not in ventricular trabeculae. Adrenomedullin did not affect contractility in either atrial or ventricular trabeculae. In conclusion, these results suggest that α-CGRP has a positive inotropic effect that can be mediated by both $CGRP_1$ and $CGRP_2$ receptors. Amylin seems to have a potential positive inotropic effect on atrial tissue, whereas no direct effect of adrenomedullin could be measured.

Keywords: Amylin; Adrenomedullin; (CGRP) Calcitonin gene-related peptide; Myocardial contractility; Inotropic effect

1. Introduction

The cardiovascular system is under the influence of nervous impulses that may affect heart rate and contractility directly or indirectly, in order to maintain an adequate circulation during various (patho) physiological conditions. Heart failure is associated not only with enhanced sympathetic tone and diminished parasympathetic tone (Porter et al., 1990), but also with increased plasma levels of calcitonin gene-related peptide (CGRP) (Ferrari et al., 1991) and adrenomedullin (Jougasaki et al., 1995b). Apart from the indirect effect these neurohumoral changes can have on the function of the heart, mainly through the modulation of peripheral resistance, there are indications that CGRP, and perhaps also the related peptides amylin and adrenomedullin, may have a direct effect on cardiac contractility (Gennari et al., 1990; Bell and McDermott, 1995; Parkes and May, 1997).

CGRP (Amara et al., 1982) is released from peripheral sensory nerve endings, and has been demonstrated both in association with the coronary vasculature and myocardial cells (Franco-Cereceda et al., 1987c; Wharton and Gulbenkian, 1989; Gulbenkian et al., 1993; Saetrum Opgaard et al., 1995). CGRP appears in two isoforms, α- and β-CGRP, with similar biological activities (Morris et al., 1984; Amara et al., 1985), and CGRP mediates its effect via at least two functional receptor subtypes, the $CGRP_1$ and the $CGRP_2$ receptor (Dennis et al., 1991; Wimalawansa, 1996). CGRP is a potent vasorelaxant that causes fall in blood pressure, increased heart rate and positive inotropic effect when administered intravenously in healthy volunteers (Gennari and Fischer, 1985; Franco-Cereceda et al., 1987b). It is not 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 direct positive inotropic effect of CGRP, or an effect only on the atria and not on the ventricles (Sigrist et al., 1986; Franco-Cereceda et al., 1987a; Ishikawa et al., 1987; Raddino et al., 1997).

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Amylin has considerable homology with CGRP (Westermark et al., 1986; Cooper et al., 1987) and is also present, among other tissues, 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). Little is known about the effect of amylin on cardiac contractility, but amylin has been demonstrated to act via CGRP receptors in mammalian myocardial tissue (Giuliani et al., 1992; Bell and McDermott, 1995; Bell et al., 1995).

Adrenomedullin has structural similarities to CGRP and amylin (Kitamura et al., 1993) and seems to act via CGRP receptors in some tissues (Zimmermann et al., 1996; Poyner, 1997). Adrenomedullin is abundantly present in the mammalian heart (Sakata et al., 1993, 1994; Jougasaki et al., 1995a). Adrenomedullin has strong hypotensive properties (Nakamura et al., 1995; Parkes, 1995). Positive hemodynamic effects of adrenomedullin have been demonstrated in vivo in healthy conscious sheep (Parkes, 1995; Parkes and May, 1997) as well as in sheep with pacing-induced heart failure (Rademaker et al., 1997), but as to the direct effect of adrenomedullin on cardiac contractility, there is very limited information.

The aim of the present study was to assess the effects of α-CGRP, amylin, and adrenomedullin on contractile force of porcine isolated atrial and ventricular trabeculae. To assess the functional role of the $CGRP_1$ receptor, we used the selective $CGRP_1$ receptor antagonist α-CGRP-(8-37), which lacks seven terminal amino acid residues compared to α-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 (Cys-ACM-CGRP), which is a linear analogue of CGRP (Dennis et al., 1989).

2. Materials and methods

2.1. Functional experiments measuring myocardial contractions

The hearts came from 16 female pigs (2–3 months old, 12–14 kg) of the Yorkshire landrace, which had been used for in vivo experiments involving 5-HT receptor ligands. After sacrificing, the hearts were removed and immediately 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. Myocardial trabeculae were excised from the inner surface of the right atria and the left ventricles. 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. Resting tension was set to approximately 750 mg for atrial trabeculae and 1950 mg for ventricular trabeculae. The trabeculae were paced at 1.5 Hz using field stimulation (5 ms, voltage 20% above threshold for contractile response), through electrodes placed in the organ baths. During continuous pacing, the tissues were allowed to stabilise for approximately 90 min before the baseline contractile amplitude was measured. Concentration–response curves for noradrenaline were obtained in some 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 each trabeculae and for comparison with other positive inotropic agents. Trabeculae that responded with an increase in contractile force of less than 25 mg to 10^{-5} M noradrenaline were excluded from the study. After several wash outs with normal Krebs buffer and stabilisation at baseline contractile force, cumulative concentrations (10^{-11} to $3 \cdot 10^{-7}$ M) of agonist were added and changes in contractile force observed. α-CGRP was also tested after 15 min preincubation with the $CGRP_1$ receptor antagonist, α-CGRP-(8-37) (10^{-6} M). At the end of the experiments, the reactivity of the trabeculae was again tested by exposure to noradrenaline (10^{-5} M). Comparison of responses to different agonists (α-CGRP, CysACM-CGRP, amylin and adrenomedullin) as well as comparison of responses to α-CGRP with and without preincubation with α-CGRP-(8-37) were performed in different trabeculae, thereby avoiding eventual desensitisation of specific receptors.

2.2. Analysis of data

Maximum increase in contractile amplitude obtained with agonist (E_{max}) and the negative logarithm of the concentration of agonist that elicited half-maximum effect (pD_2) were derived from concentration–response curves on each trabecula. As the size of the trabeculae could vary, the agonist E_{max} value was calculated as percentage of baseline contractile amplitude, as well as percentage of increase in contractile amplitude induced by noradrenaline (10^{-5} M) in the same trabecula. Values are given as mean ± S.E.M. Comparison of responses in different trabeculae were performed with one-factor analysis of variance (ANOVA) followed by post-hoc analysis using the Bonferroni/Dunn (All Means) test. When comparing agonist-induced response to baseline contractile amplitude in the same trabecula, Student's paired *t*-test was used. Statistical significance was assumed when $P < 0.05$.

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] (CysACM-CGRP; Peninsula, St. Helens, UK). The drugs were dissolved in distilled water.

3. Results

3.1. Baseline contractile amplitude and response to noradrenaline

Concentration–response curves for noradrenaline up to a concentration of 10^{-4} M were performed on eight atrial and eight ventricular trabeculae. The baseline contractile force (mean ± S.E.M.) was 150 ± 25 mg in the atrial and 428 ± 155 mg in the ventricular trabeculae. The contractile amplitude was significantly stronger in ventricular compared to atrial trabeculae ($P < 0.05$), which can be explained by differences in trabecular size. The E_{max} values for noradrenaline were 145 ± 21% in atrial and 60 ± 11% in ventricular trabeculae; these values representing mean ± S.E.M. of increase in contractile force measured as percentage of baseline contractile amplitude in each individual trabecula. The E_{max} values for noradrenaline were significantly higher in atrial compared to ventricular tissue ($P < 0.05$). The pD_2 values for noradrenaline were similar, 6.29 ± 0.21 in atrial and 6.09 ± 0.28 in ventricular trabeculae.

The concentration–response curves for noradrenaline showed that a concentration of 10^{-5} M gave a nearly maximum response to noradrenaline in both atrial and ventricular trabeculae, and this concentration of noradrenaline was used to assess the responsiveness of the trabeculae prior to testing different agonists.

When comparing responses to different agonists, the values of baseline contractile amplitude, as well as absolute values for increase in contractile amplitude induced by noradrenaline showed rather large variations between different treatment groups (Table 1). Although there are significant differences between atrial and ventricular groups, similar to what was reported above, the differences among atrial groups separately, and among ventricular groups separately, did not turn out significant when multiple comparison analysis was performed.

3.2. α-CGRP

α-CGRP increased contractile force in 5 out of 10 atrial trabeculae tested and in 3 out of 7 ventricular trabeculae tested (Table 1, Figs. 1 and 2). In both atrial and ventricular trabeculae, the contractile amplitudes after exposure to α-CGRP were significantly higher than baseline contractile

Table 1
Positive inotropic effect of α-CGRP without and after preincubation with α-CGRP-(8-37) (10^{-6} M), and the effect of CysACM-CGRP (CysACM), amylin and adrenomedullin (ADM) tested on isolated porcine trabeculae from right atria and left ventricles
n_1 = number of trabeculae tested, n_2 = number of trabeculae responding to agonist. Baseline (mg) = contractile amplitude before exposure to agonist, measured in milligrams. Increase NA (mg) = contractile amplitude after exposure to noradrenaline (10^{-5} M) minus baseline contractile amplitude, measured in milligrams. All agonists were tested at baseline contractile force, and in order to better assess a possible negative inotropic effect, CysACM-CGRP and adrenomedullin (ADM) were also tested on trabeculae stimulated with noradrenaline (NA) (10^{-5} M) (= CysACM-CGRP with NA and ADM with NA), but with no measurable effect. E_{max} (% of BL) = maximum increase in contractile amplitude induced by agonist, measured as percentage of baseline contractile amplitude in the same trabecula. E_{max} (% of NA) = maximum increase in contractile amplitude induced by agonist, measured as percentage of increase in contractile amplitude induced by NA (10^{-5} M) in the same trabecula. The calculations are done on each individual trabecula, and values given as mean ± S.E.M. For E_{max} values, only trabeculae responding to agonist are included in the calculation, whereas Baseline (mg) and Increase NA (mg) represent all tested trabeculae in that group. α-CGRP-(8-37) (10^{-6} M) completely blocked the effect of α-CGRP in atrial trabeculae, and almost completely blocked the effect of α-CGRP in ventricular trabeculae.

Agonist	n_1	n_2	Baseline (mg)	Increase NA (mg)	E_{max} (% of BL)	E_{max} (% of NA)	pD_2
Atria							
CGRP	10	5	168 ± 30	231 ± 69	34 ± 11	15 ± 2	8.22 ± 0.15
CGRP-(8-37) + CGRP	7	0	174 ± 33	260 ± 48	0	0	
CysACM	5	2	87 ± 12	175 ± 43	33 ± 9	14 ± 7	7.88 ± 0.12
CysACM with NA	5	0	129 ± 34	269 ± 44	0	0	
Amylin	5	2	127 ± 31	165 ± 50	17 ± 11	10 ± 3	7.55 ± 0.79
ADM	6	0	111 ± 27	146 ± 47	0	0	
ADM with NA	9	0	178 ± 37	286 ± 30	0	0	
Ventricles							
CGRP	7	3	384 ± 93	151 ± 42	18 ± 7	41 ± 13	8.67 ± 0.16
CGRP-(8-37) + CGRP	7	1	336 ± 101	195 ± 74	4	8	7.51
CysACM	5	1	269 ± 25	113 ± 26	7	15	8.51
CysACM with NA	4	0	338 ± 137	228 ± 42	0	0	
Amylin	5	0	489 ± 116	226 ± 37	0	0	
ADM	6	0	307 ± 59	103 ± 26	0	0	
ADM with NA	8	0	474 ± 115	381 ± 87	0	0	

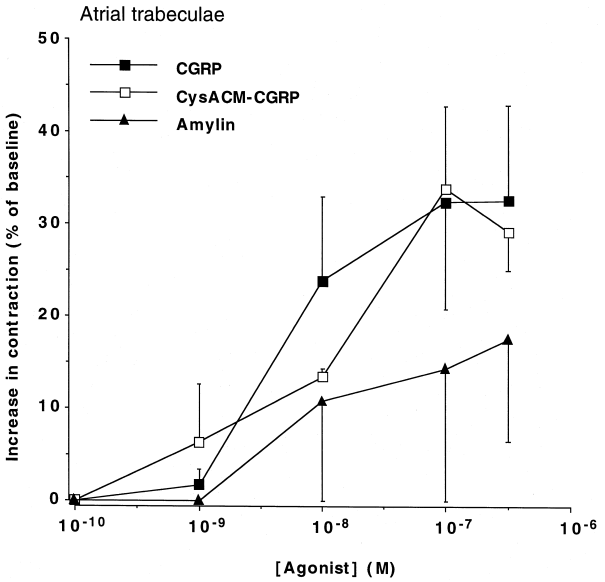

Fig. 1. Comparison of positive inotropic effects in porcine atrial trabeculae responding to α-CGRP (5 responding out of 10 tested trabeculae), the $CGRP_2$ receptor agonist CysACM-CGRP (2 responding out of 5 tested trabeculae) and amylin (2 responding out of 5 tested trabeculae). Preincubation with the $CGRP_1$ receptor antagonist α-CGRP-(8-37) (10^{-6} M) completely blocked positive inotropic responses to α-CGRP. Adrenomedullin had no inotropic effects. 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.

amplitudes ($P < 0.05$). When the increase in contractile force was measured as percentage of baseline contractile force in the same trabecula, the E_{max} value for α-CGRP was higher in atrial compared to ventricular trabeculae, but this difference was not significant. However, due to differences in noradrenaline-induced responses between atrial and ventricular trabeculae, the E_{max} value for α-CGRP was higher in ventricular than in atrial trabeculae when measured as percentage of noradrenaline-induced response in the same trabecula, but this difference was not significant either. The pD_2 value in atrial trabeculae (8.22 ± 0.15) was not significantly different from that in ventricular trabeculae (8.67 ± 0.16), (for further details, see Table 1, Figs. 1 and 2).

3.3. α-CGRP-(8-37)

After preincubation with the $CGRP_1$ receptor antagonist α-CGRP-(8-37) (10^{-6} M), cumulative concentrations of α-CGRP (10^{-11} M to $3 \cdot 10^{-7}$ M) did not cause any increase in contractile force in any of the atrial trabeculae ($n = 7$), and a weak response was seen in only 1 out of 7 ventricular trabeculae tested (Table 1 and Fig. 2).

3.4. CysACM-CGRP

The $CGRP_2$ receptor agonist CysACM-CGRP tested in cumulative concentrations from 10^{-11} M to $3 \cdot 10^{-7}$ M had a positive inotropic effect in 2 out of 5 atrial and in 1 out of 5 ventricular trabeculae tested. The E_{max} and pD_2 values were not significantly different from those of α-CGRP, but due to the small number of responding trabeculae, a significant increase in contractile amplitudes after exposure to CysACM-CGRP compared to baseline could not be established. To be able to better determine a possible negative inotropic effect, CysACM-CGRP was also tested on trabeculae precontracted with noradrenaline (10^{-5} M), but no changes in contractile force in either atrial ($n = 5$) or ventricular ($n = 4$) trabeculae were observed (Table 1, Figs. 1 and 2).

3.5. Amylin

Amylin (10^{-11} M to $3 \cdot 10^{-7}$ M) had a positive inotropic effect in 2 out of 5 atrial trabeculae but in none of the ventricular trabeculae tested ($n = 5$). The E_{max} and pD_2 values were not significantly different from those of α-CGRP or CysACM-CGRP, although amylin tended to have lower E_{max} and pD_2 values (Table 1 and Fig. 1). Similar to CysACM-CGRP, due to the few responding trabeculae, a significant difference between baseline contractile amplitude and contractile amplitude after exposure to amylin could not be calculated.

3.6. Adrenomedullin

Adrenomedullin (10^{-11} M to $3 \cdot 10^{-7}$ M) did not influence the baseline contractile force of either atrial ($n = 6$)

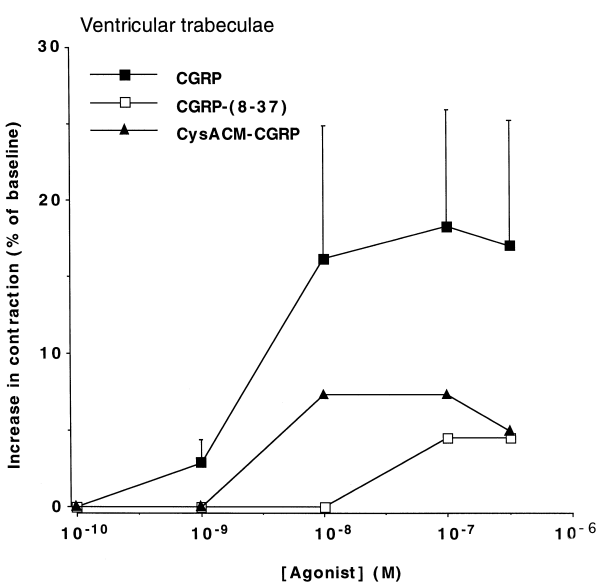

Fig. 2. Comparison of positive inotropic effects in porcine ventricular trabeculae responding to α-CGRP (3 responding out of 7 tested trabeculae), α-CGRP after preincubation with α-CGRP-(8-37) (10^{-6} M) (1 responding out of 7 tested trabeculae) and CysACM-CGRP (1 responding out of 5 tested trabeculae). Amylin and adrenomedullin had no inotropic effects. Each point represents the mean of agonist responses calculated as percentage of baseline contractile amplitude and only including responding trabeculae. The S.E.M are shown as vertical bars.

or ventricular ($n = 6$) trabeculae. Similar to CysACM-CGRP, adrenomedullin was also tested in cumulative concentrations from 10^{-11} M to $3 \cdot 10^{-7}$ M on trabeculae stimulated with noradrenaline at a concentration of 10^{-5} M. Even here no changes in contractility were seen in either atrial ($n = 9$) or ventricular ($n = 8$) trabeculae (Table 1).

4. Discussion

4.1. Positive inotropic effects mediated by CGRP receptors

In the present experiments, we demonstrate that α-CGRP can have a direct positive inotropic effect at both the atrial and the ventricular level. The antagonistic effect of the $CGRP_1$ receptor antagonist α-CGRP-(8-37) suggests that $CGRP_1$ receptors mediate positive inotropic responses in both atrial and ventricular trabeculae. The $CGRP_2$ receptor agonist CysACM-CGRP had a clear positive inotropic effect in some trabeculae. This suggests that even $CGRP_2$ receptors have the potential to mediate positive inotropic responses, although this could not be statistically proven due to the limited number of responding trabeculae. From our results, it is however questionable if this receptor subtype has any functional importance as mediator of positive inotropic responses in ventricular tissue. In a previous study on isolated rat ventricular cardiomyocytes, CGRP had a positive inotropic effect that was antagonised by CGRP-(8-37), and also CysACM-CGRP had a contractile effect (Bell and McDermott, 1994). In electrically driven isolated guinea pig left atria, however, CysACM-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). Competitive receptor binding studies in rat atrium actually demonstrated that α-CGRP had the highest affinity to what appeared to be CGRP receptors, followed by α-CGRP-(8-37), CysACM-CGRP and finally amylin (Van Rossum et al., 1994). Differences in previous studies and the fact that CysACM-CGRP as well as CGRP in the present study had a positive inotropic effect in only some of the trabeculae, could thus have its explanation in different density, or functional state of specific receptors, or second messenger mechanisms. Furthermore, it was recently proposed that receptor specificity of the previously cloned $CGRP_1$ receptor (Aiyar et al., 1996), which has a cDNA sequence identical to that of a calcitonin receptor-like receptor, is conferred by receptor associated modifying proteins (RAMPs), which are required to transport the calcitonin receptor-like receptor to the plasma membrane (McLatchie et al., 1998).

4.2. Amylin

In our study, amylin had a positive inotropic effect in a few atrial trabeculae, but none in the ventricular. The responses were clear in the responding trabeculae, but as only a few trabeculae reacted to amylin, these responses could not be statistically proven. Our results are in concert with a previous study on isolated guinea-pig left atrium, where amylin had a positive inotropic effect presumably mediated via $CGRP_2$ receptors, but with lower potency than that of CGRP (Giuliani et al., 1992). The fact that amylin had no effect on ventricular tissue in our study, does not exclude that amylin can have the potential to increase contractility also of ventricular tissue. It has actually been shown that amylin has a positive inotropic effect possibly mediated via $CGRP_1$ receptors in rat ventricular cardiomyocytes, but less potent than that of CGRP (Bell and McDermott, 1995). CGRP and amylin have further been shown to exert hypertrophic effects on ventricular cardiomyocytes from adult rats, probably mediated via $CGRP_1$ receptors at which amylin did bind with lower potency than CGRP (Bell et al., 1995). Comparison of potencies between amylin, α-CGRP, and CysACM-CGRP in the present study was not meaningful due to the limited number of responding trabeculae.

4.3. Adrenomedullin

We could not detect any inotropic effects of adrenomedullin, either positive or negative, on atrial or ventricular trabeculae, which makes it less likely that adrenomedullin has any major function as direct regulator of cardiac contractile force. On the other hand, variations between different trabeculae for responses to other agonists in the present study show that a potential inotropic effect of an agonist is not necessarily detected in all trabeculae in this experimental setting. It can therefore not be excluded that adrenomedullin possesses weak inotropic effects that could not be measured in our experiments as the conditions might not have been optimal. Previous studies demonstrating increased levels of adrenomedullin in plasma (Jougasaki et al., 1995b; Nishikimi et al., 1995) and myocardium (Jougasaki et al., 1995b) of heart failure patients and reports that the failing human heart secretes adrenomedullin (Jougasaki et al., 1996; Nishikimi et al., 1997), suggest a role of this peptide in the cardiac regulation. Previous in vivo studies on laboratory animals have shown positive hemodynamic effects of adrenomedullin with increased cardiac output, but it seems unclear to what extent this represents a direct positive inotropic effect or reflex changes due to a lowering of the blood pressure (Parkes, 1995; Parkes and May, 1997; Rademaker et al., 1997). Regarding in vitro studies, it has been reported that adrenomedullin has a positive inotropic effect on isolated rat hearts (Szokodi et al., 1996, 1998), but a negative inotropic effect on isolated rabbit ventricular myocytes (Ikenouchi et al., 1997). These discrepancies could eventually have its explanation if adrenomedullin interferes with CGRP receptors or other receptors mediating inotropic

effects. Whereas adrenomedullin seems to act via CGRP-like receptors in some tissues (Zimmermann et al., 1996), separate receptors seem to be involved in other tissues (Kato et al., 1995). Specific binding sites for adrenomedullin have been detected in rat hearts, where both adrenomedullin, amylin, and CGRP all competed with [^{125}I]adrenomedullin binding (Owji et al., 1995). It was newly proposed that adrenomedullin may act via $CGRP_1$ receptors activated by a specific RAMP conferring receptor specificity for adrenomedullin (McLatchie et al., 1998). Looking at previous data, it is thus possible that adrenomedullin has a functional role in the regulation of cardiac contractility at least during certain pathophysiological conditions. The binding of adrenomedullin to specific receptors, on the other hand, does not necessarily imply the initiation of positive inotropic effects.

4.4. Positive inotropic effect in atrial vs. ventricular tissue

In the present study, the E_{max} values for noradrenaline when performing dose–response curves were significantly higher in atrial compared to ventricular tissue, measured as percentage of baseline contractile amplitude, suggesting a higher reactivity in atrial than in ventricular tissue. CGRP and CysACM-CGRP also tended to have stronger positive inotropic effects in atrial compared to ventricular tissue, measured as percentage of baseline contractile amplitude, although the differences were not statistically different, and amylin had an effect only in atrial trabeculae. Although statistically not proven, our results might suggest, as discussed above for the $CGRP_2$ receptor and amylin, that CGRP has a more important role in the atria than in the ventricles. On the other hand, due to the limited number of responding trabeculae, differences in E_{max} and pD_2 values should be interpreted cautiously. Furthermore, when comparing agonist responses, our data show (Table 1) that there are rather large variations in the responses to noradrenaline between different treatment groups, although significant differences in noradrenaline-responses were seen only between atrial and ventricular groups, and not among atrial groups or among ventricular groups. These differences in noradrenaline-induced responses between different trabeculae could have implications when comparing responses to other agonists in different trabeculae, but ought to be of minor importance as agonist responses are measured as percentage of baseline contractile amplitude and as percentage of response induced by noradrenaline. Furthermore, rather than comparing absolute values of noradrenaline-induced increase in contractile response, these values should be related to baseline contractile amplitude in each treatment group, and variations in baseline contractile amplitudes can be explained by differences in the size of the trabeculae. The responses to noradrenaline were generally stronger in atrial than in ventricular trabeculae when compared to baseline amplitude, which resulted in different relations between agonist E_{max} values in atrial vs. ventricular trabeculae depending if the E_{max} value was calculated as percentage of baseline contractile amplitude or as percentage of increase in contractile amplitude induced by noradrenaline.

Previous studies have demonstrated a positive inotropic effect of CGRP in the right atrium of isolated rat hearts (Sigrist et al., 1986) and in the isolated, electrically driven auricle of the human right atrium (Franco-Cereceda et al., 1987a), whereas no inotropic effects were seen in isolated rabbit hearts (Raddino et al., 1997). Furthermore, CGRP had a positive inotropic effect in isolated atrial but not in ventricular muscles from rat (Ishikawa et al., 1987), while no effects were seen in either isolated atrial or ventricular muscles from dog (Rigel et al., 1989). A differential role of CGRP in the atria compared to the ventricles has also been proposed from studies in guinea-pigs and humans demonstrating four-fold higher levels of CGRP-like-immunoreactivity in the atria than in the ventricles (Franco-Cereceda et al., 1987c). Furthermore, there is an abundance of high affinity binding sites for CGRP in the atria of laboratory animals, but relatively little in the ventricles (Sigrist et al., 1986; Van Rossum et al., 1994; Rubino and Burnstock, 1996). CGRP has previously been assumed to be physiologically important as an inotropic agent only in the atria, and it has been proposed that the positive inotropic effect of CGRP seen in vivo is of reflex origin due to the concomitant fall in blood pressure (Franco-Cereceda et al., 1987b; Ishikawa et al., 1987; Du et al., 1994). Interestingly though, it has been demonstrated that infusion of CGRP into patients with congestive heart failure improved myocardial contractility without any consistent change in arterial pressure or heart rate (Gennari et al., 1990). Furthermore, in a study on porcine tissue, CGRP had a positive inotropic effect only on the trabeculae from the left ventricle and not on those from the right atrium (Van Gelderen et al., 1995), and CGRP had a positive inotropic effect on isolated rat ventricular cardiomyocytes (Bell and McDermott, 1994). Contrary to these findings, CGRP had a negative inotropic effect on isolated rabbit cardiac ventricular myocytes (Ikenouchi et al., 1997). The diversity in previous results from in vitro experiments could depend on species differences or experimental conditions. Our study, however, does show that CGRP has the potential to exert a positive inotropic effect at both the atrial and ventricular level of porcine hearts. CGRP thus appears to influence cardiac contractility, not only indirectly through its vasorelaxant effect, but also through a direct effect on the heart. This together with the previously demonstrated elevation of CGRP plasma-levels in congestive heart failure (Ferrari et al., 1991) suggests an important role of this peptide in heart failure patients.

4.5. Conclusion

In the present study, α-CGRP had a potent positive inotropic effect in nearly half of the trabeculae tested, and

the effect of α-CGRP was almost completely blocked after preincubation with the $CGRP_1$ receptor antagonist α-CGRP-(8-37) (10^{-6} M). The $CGRP_2$ receptor agonist CysACM-CGRP had a positive inotropic effect in only some trabeculae, and the effect seemed to be negligible in ventricular trabeculae. Amylin had a positive inotropic effect in only some atrial trabeculae, but in none of the ventricular trabeculae, and adrenomedullin had no inotropic effects in either atrial or ventricular tissue.

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