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The effect of adrenomedullin, amylin fragment $_{8–37}$ and calcitonin gene-related peptide on contractile force, heart rate and coronary perfusion pressure in isolated rat hearts

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The effect of human adrenomedullin, human amylin fragment $_{8–37}$ (amylin $_{8–37}$) and rat calcitonin gene-related peptide (CGRP) on contractile force, heart rate and coronary perfusion pressure has been investigated in the isolated perfused rat hearts.

Adrenomedullin (2×10^{-10}, 2×10^{-9} and 2×10^{-8} M) produced a significant decrease in contractile force and perfusion pressure, but only the peptide caused a decline in heart rate at the highest dose. Amylin (10^{-9}, 10^{-8} and 10^{-7} M) significantly increased and then decreased contractile force. Two doses of amylin (10^{-8} and 10^{-7} M) induced a significant increase in heart rate, however amylin did not change perfusion pressure in all the doses used. Rat alpha CGRP (10^{-8}, 10^{-7} and 10^{-6} M) evoked a slight decline in contractile force following a significant increase in contractile force induced by the peptide. CGRP in all the doses raised heart rate and lowered perfusion pressure.

Our results suggest that adrenomedullin has negative inotropic, negative chronotropic and coronary vasodilator actions. Amylin produces a biphasic inotropic effect and evokes a positive chronotropy. CGRP causes positive inotropic, positive chronotropic and vasodilatory effects in isolated rat hearts.

Keywords: adrenomedullin, amylin fragment $_{8–37}$, calcitonin gene-related peptide (CGRP), isolated perfused rat heart, contractile force

Adrenomedullin (AM) which was firstly found in human pheochromocytoma tissue is a peptide and exerts a potent vasorelaxant action (24). The peptide is a member of calcitonin gene-related peptide (CGRP) family and contains 52 amino acids in human

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and 50 amino acids in rat (43, 49). Immunoreactivity of AM has been demonstrated to exist in various tissues such as adrenal medulla, heart, lung and human plasma (17, 24). The production of AM has also been demostrated in cardiac myocytes (6), vascular endothelial cells (53) and smooth muscle cells (54). These observations suggest that AM may function as an endogenous regulator in the control of regional vascular resistance. The findings from *in vivo* experiments also indicate that AM evokes hypotension and increases cardiac output, heart rate and left ventricular contractility (37). In addition, AM produces diuresis, natriuresis (7, 21) and inhibits water intake and salt appetite (20). AM also inhibits the secretion of aldosteron from adrenal cortex (47), endothelin-1 from vascular endothelial cells (27), adrenocorticotropin release from the anterior pituitary gland (34), apoptosis in endothelial cells (48) and the proliferation of vascular smooth muscle (23). Thus, the peptide may play an important role in the regulation of cardiovascular and renal function.

A 37 amino acid peptide amylin which possesses major sequence homology with CGRP (8) is produced in beta cells of pancreatic islets of Langerhans (3). Amylin interacts with CGRP receptors (12, 40) and also with amylin receptors (12, 39). Amylin decreases second phase insulin secretion and increases hepatic glucose output (8). Amylin has been reported to have vasodilator activity (8). In addition it has been reported that amylin causes tachycardiac and hypotensive effects in rats (10).

CGRP is a 37 amino acid neuropeptide resulting from an alternate splicing of the calcitonin gene (1, 44). Two forms of CGRP termed α and β are synthetised in both rat and human, and the biological activities of these forms are very similar to each other (41). CGRP is known to be widely distributed in both central and peripheral nervous system in mammals (60), but also found in many other tissues (42). CGRP-like immunoreactivity or binding sites for CGRP have also been shown in blood vessels and heart tissues including coronary arteries, atria and ventricular myocardium (33, 50, 51, 58). CGRP exerts a positive inotropic effect on isolated human (36) and porcine (35) myocardial trabeculae. Therefore, it has been suggested that $CGRP_1$ and $CGRP_2$ receptors may mediate the positive inotropic effect (35, 36). Stimulation of perivascular sensory nerves provokes CGRP release and the peptide produces a powerful vasodilatory effect on venous and arterial vessels (41). Furthermore, CGRP has a positive chronotropic effect on the heart (42). These findings indicate that CGRP has a physiological role in the cardiovascular regulation. However, conflicting results concerning the effects of CGRP and AM have appeared in the literature. Franco-Cereceda and Lundberg (9), Tippins et al. (57) and Lundberg et al. (28) showed that CGRP produced a stimulatory effect on cardiac contractility. In contrast, Manzini et al. (29) demonstrated a negative inotropic effect. In isolated rat hearts Szokodi et al. (55, 56) reported that AM induced a positive inotropic effect on heart contractility. However, Ikenouchi et al. (18) demonstrated that AM caused a negative inotropic effect in isolated rabbit cardiac myocytes. Perret et al. (38) also suggested that AM had a negative inotropic activity in isolated rat hearts. Furthermore, in the isolated heart preparations the effect of a 30 amino acid peptide amylin fragment $_{8–37}$ on contractile force, heart rate and coronary vascular tone has not been examined yet. In the present

study we have therefore investigated the effects of AM, amylin $_{8–37}$ and CGRP on contractile force as well as heart rate and coronary perfusion pressure in the isolated rat hearts.

Materials and Methods

Animals

Sprague–Dawley rats of either sex weighing between 350–450 g were used in all the experiments. Animals were obtained from Animal Unit of Physiology Department established in 1986 and the investigation was made in accord with the principles outlined in the Declaration of Helsinki (Cardiovasc. Res., 1997; 35: 2–3).

Preparation

One hour after the administration of 1000 IU heparin i.p., the chest was opened under light ether anesthesia, and the heart was excised rapidly and then placed into the ice-cold (0–4 °C) modified Krebs-Henseleit solution (mKHs) until contractions ceased. After the heart was cleaned off surrounding fat and other tissues, aorta was immediately tied to a stainless steel perfusion cannula and heart was perfused retrogradely by the nonrecirculating Langendorff technique. The pulmonary artery was incised to facilitate complete coronary drainage in the ventricles.

Materials

The perfusion solution was daily prepared mKHs with following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11. mKHs was continuously oxygenated with 95% O_2 and 5% CO_2 using a disposable infant oxygenator (Dideco Masterflo, Model D701, Mirandola) and pH of the solution was 7.4. The temperature was continuously measured in aortic cannula and maintained at 37 °C. The hearts were perfused under constant flow condition (10 ml/min) using an infusion pump (LifeCare®Pump, Abbott/Shaw, Model 4, Chicago).

Recording

For the measurement of the force of contraction, a metal hook was attached to the apex of the heart and connected to a force displacement transducer (TB 611T, Nihon Kohden, Tokyo). Cardiac contractile force was recorded on the recorder (WI 641G, Nihon Kohden, Tokyo) of a polygraph (RM 6000, Nihon Kohden, Tokyo). Heart rate was calculated from the polygraph tracings of the contractile force at a paper speed of 2.5 mm/s. Coronary perfusion pressure was measured by attaching a side arm of the aortic cannula to a pressure transducer (TB 200T, Nihon Kohden,Tokyo). The hearts were equilibrated for 30 min to establish a stable baseline.

Experimental protocol

In the first group of the experiments, human amylin fragment $_{8-37}$ (10^{-9},10^{-8} and 10^{-7} M, n=8, Sigma) and in the second group of the experiments rat alpha CGRP (10^{-8}, 10^{-7} and 10^{-6} M, n=6, Sigma) were injected in a bolus fashion (100 µl) into the perfusate, 2 cm proximal to the aortic cannula. In the third group by means of an infusion pump (Graseby Medical, Model 3400, Watford Herts) human AM (2×10^{-10}, 2×10^{-9} and 2×10^{-8} M, n=6, Sigma) was infused into the side arm of the perfusion cannula at a rate of 0.5 ml/min for 5 min. The effect of increasing doses was evaluated in a noncumulative manner. Before starting the experiments a resting tension of 2 was applied to each heart to yield an optimal contractile force. The recovery time of cardiovascular effects of the peptides after each dose was 5 min.

Statistics

Values are given as mean ± S.E.M. The values obtained before the addition of drugs were taken as controls. Statistical analysis of the data was performed by two-way analysis of variance (ANOVA) followed by the Tukey–HSD multiple comparisons test. A p value less than 0.05 was considered to be significant.

Results

The infusion of AM (2×10^{-10}, 2×10^{-9} and 2×10^{-8} M) decreased ($p<0.05$) contractile force (n=6). The maximal decrease in contractility occurred 1 min after the infusion of the peptide which caused 8, 18 and 20% decreases in contractile force, respectively (Fig. 1). Low doses of AM (2×10^{-10} and 2×10^{-9} M) produced a slight and insignificant decline in heart rate (Fig. 2). However, the highest dose of peptide (2×10^{-8} M) resulted in about 6% reduction ($p<0.05$) in heart rate after 5 min from administration. Furthermore, after 1–2 min of the infusion AM with the same three doses (Fig. 3) also lowered perfusion pressure by 9, 15 and 17%, respectively ($p<0.05$).

The bolus injections of amylin $_{8-37}$ produced a biphasic change in cardiac contractile force with an initial increase and then a subsequent decrease. Amylin (10^{-9}, 10^{-8} and 10^{-7} M, Fig. 4) significantly increased contractile force (32, 40 and 48%, respectively) after 15–20 s from the injections ($p<0.05$). However, the same doses caused significant declines in contractile force within 3–5 min following positive inotropic effect. The lowest decreases in contractility were reached after 5 min and amylin (10^{-9}, 10^{-8} and 10^{-7} M) caused 16, 18 and 25% reductions ($p<0.05$) in contractile force, respectively (n=8). Significant increases in heart rate (Fig. 5) were obtained with the doses of 10^{-8} and 10^{-7} M (n=7) and the peak increases after 2 min were 11 and 22%, respectively ($p<0.05$). Although small decreases in perfusion pressure were observed, amylin did not statistically alter the perfusion pressure (Fig. 6).

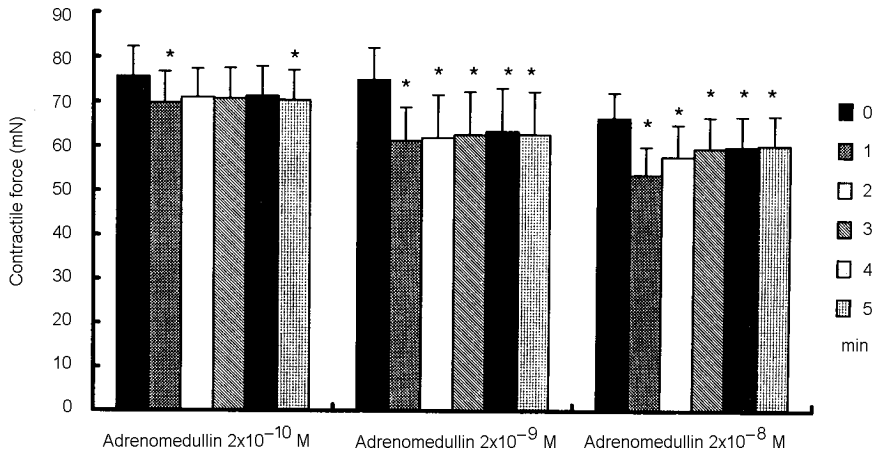

Fig. 1. Time-dependency of the changes in contractile force in response to adrenomedullin in isolated rat hearts (n=6). Time 0 corresponds control value. Vertical bars show S.E.M. Asterisks indicate statistically significant differences ($p<0.05$) from the respective controls. Two-way analysis of variance (ANOVA) followed by the Tukey–HSD multiple comparisons test

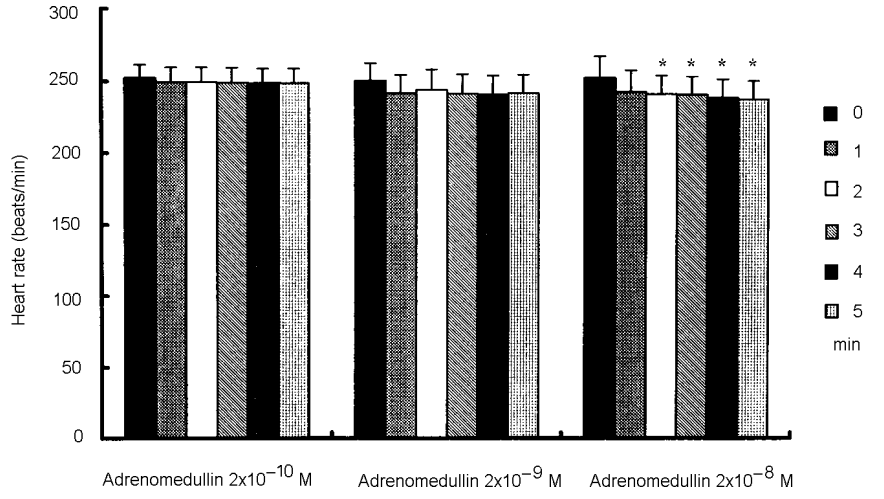

Fig. 2. Time-dependency of the changes in heart rate in response to adrenomedullin doses (n=6) (See explanation and signs as Fig. 1)

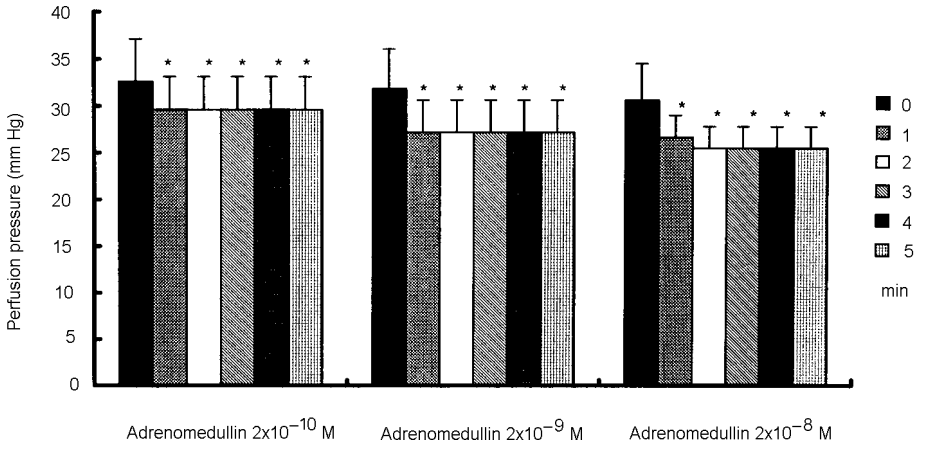

Fig. 3. The changes in perfusion pressure in response to adrenomedullin (n=6)
(See explanation and signs as Fig. 1)

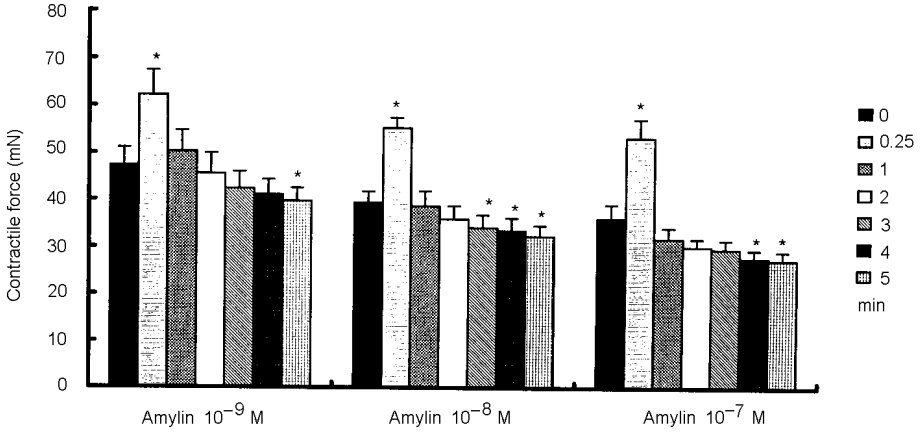

Fig. 4. Time course of the effect of amylin on contractile force (n=8) (See explanation and signs as Fig. 1)

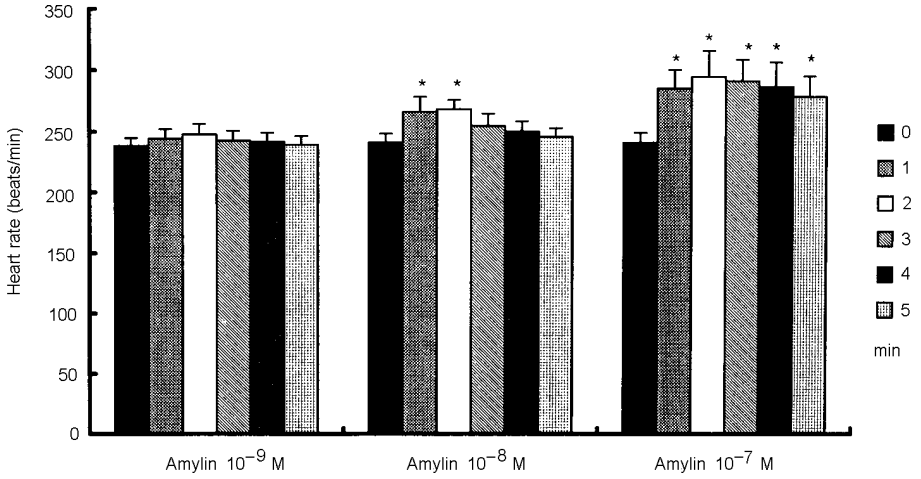

Fig. 5. The effect of amylin on heart rate (n=7) (See explanation and signs as Fig. 1)

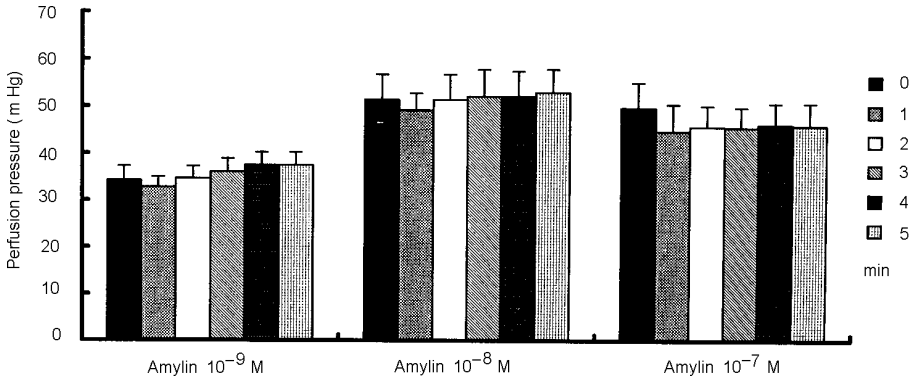

Fig. 6. The effect of amylin on perfusion pressure (n=8) (See explanation and signs as Fig. 1)

Similar to amylin treated hearts, CGRP (10^{-8}, 10^{-7} and 10^{-6} M) increased the contractile force after 15–20 s from bolus injections (Fig. 7). The peak increases (n=6) in contractile force were 42, 49 and 56% for 10^{-8}, 10^{-7} and 10^{-6} M, respectively ($p<0.05$). After the increases in contractile force, CGRP also decreased the contractile force, but these decreases were not significant. The maximal increases induced by CGRP in heart rate observed 1 min after the peptide injections were 9, 11 and 15% for

10^{-8}, 10^{-7} and 10^{-6} M, respectively ($p<0.05$, Fig. 8). CGRP decreased perfusion pressure (Fig. 9) and the maximal decreases occurred 2 min after the injections of the peptide were 22, 33 and 37% for 10^{-8}, 10^{-7} and 10^{-6} M CGRP, respectively ($p<0.05$).

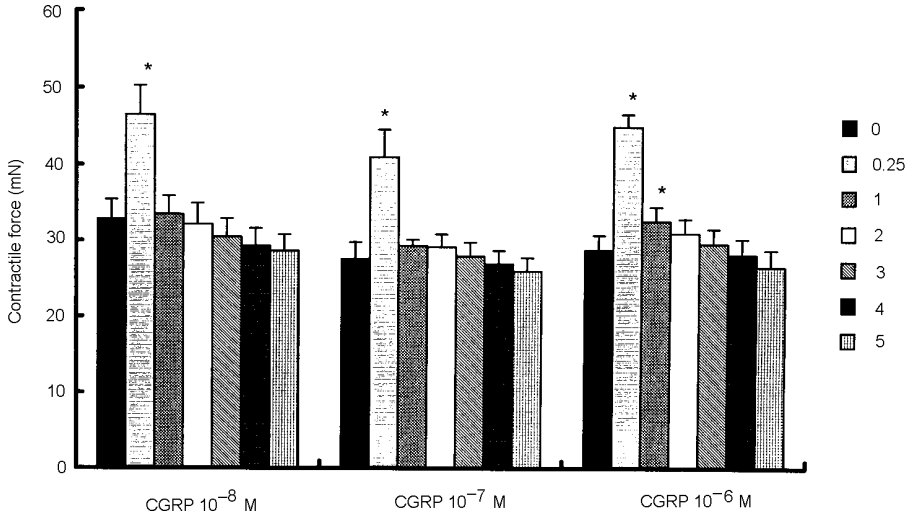

Fig. 7. Time course of the effect of CGRP on contractile force (n=6) (See explanation and signs as Fig. 1)

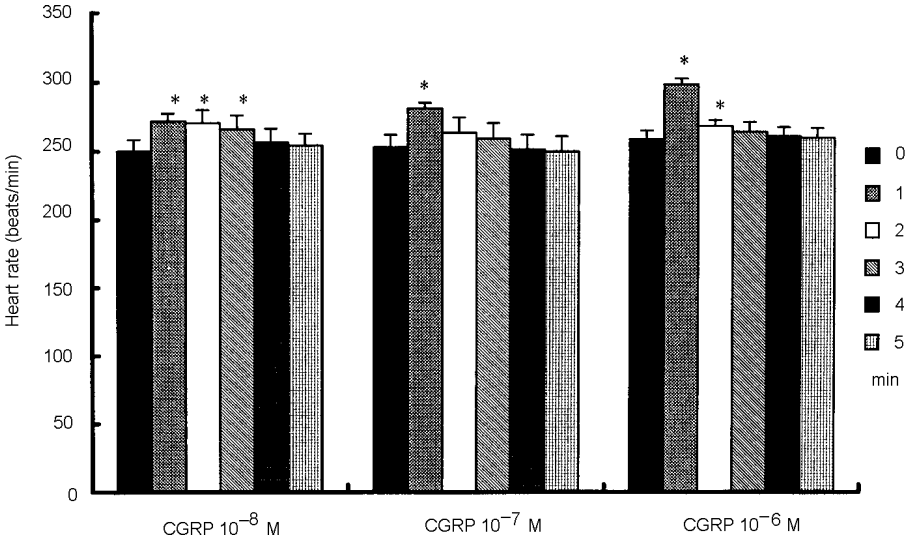

Fig. 8. The effect of CGRP on heart rate (n=6) (See explanation and signs as Fig. 1)

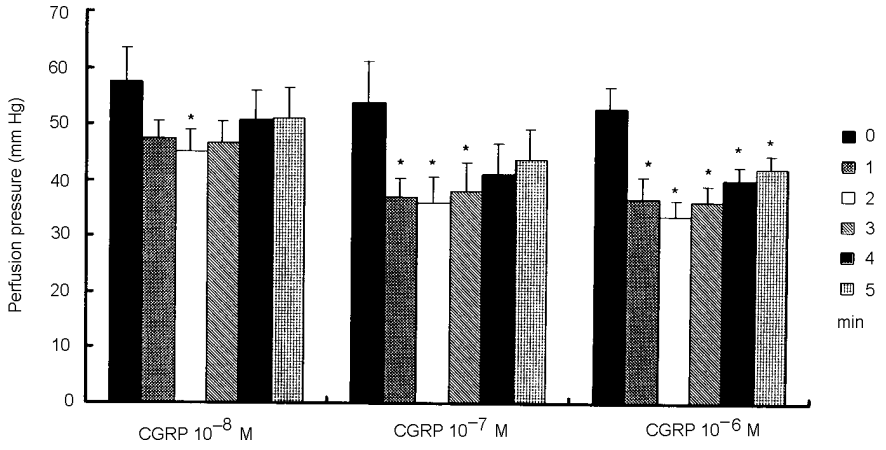

Fig. 9. The effect of CGRP on perfusion pressure (n=6) (See explanation and signs as Fig. 1)

Discussion

The present study has demonstrated that in isolated rat hearts infusion of AM causes negative inotropic effect on cardiac contractile force. In agreement with our results a negative inotropic effect of AM was previously observed in isolated rabbit cardiac ventricular myocytes (18) and isolated rat hearts (38). The negative inotropic effect of the peptide may be mediated by c-AMP-independent mechanisms, because an increase in c-AMP production in cardiomyocytes usually increases contractility. Nitric oxide (NO) may be involved in the negative inotropy. It has been suggested that the negative inotropic action of AM on cardiac myocytes may be mediated via a NO-dependent mechanism. AM increased intracellular c-GMP, which is a second messenger of NO, but not that of c-AMP (18). It has been demonstrated that NO reduces the calcium current in myocytes (31, 32, 59) which may lead to negative inotropy. Our results are different from Szokodi et al. (55, 56) who found that AM exerted a potent positive inotropic action in perfused rat hearts. These investigators used rat AM ($AM_{1–50}$) at the doses of 0.03–1 nM, while we used human AM ($AM_{1–52}$) at the doses of 0.2–20 nM. Sakata et al. (45) detected AM in normal rat tissues and observed that 6 amino acids of the rat AM are distinct from the corresponding amino acids in the human AM. The differences between the present and previous studies regarding the effects of AM on contractility may depend on the different adrenomedullins used. Human and rat AM may also affect *in vivo* cardiovascular actions differently. Human and rat AM caused hypotension in conscious rats and at the lowest dose the vasodilatory effect of rat AM was higher than that of human AM (11).

It has been observed that AM does not affect the heart rate in isolated perfused rat heart preparations (38, 55) and in anaesthetized rats (19). We have observed that although the low doses of AM did not alter heart rate, the highest dose of the peptide produced a small but significant decline in heart rate. This difference may depend on the difference of doses, because our dose is higher than that of previous studies (19, 55).

We have found that AM decreased coronary perfusion pressure, indicating coronary vasodilation. Similarly a coronary vasodilatory effect of the peptide was demonstrated in isolated rat hearts (55).

In the present study, amylin fragment $_{8–37}$ caused an initial positive and then a negative inotropic effect. The peptide also produced positive chronotropic effect and did not change the perfusion pressure. It has been demonstrated that rat amylin produced a concentration-related positive inotropic effect in the guinea-pig isolated atrium (12). Similarly, in experiments on rat ventricular cardiomyocytes amylin also increased contractile amplitude via CGRP1-preferring receptors (4). The effects of amylin fragment $_{8–37}$ on coronary vascular tone and mechanical function in isolated rat hearts have not been investigated. Our results indicate that amylin $_{8–37}$ may cause biphasic inotropic effects with positive chronotropy in isolated rat hearts. Further studies on cardiac actions of this peptide fragment are required.

The vasodilator activity of amylin amide, also known as islet amylated polypeptide, has been demonstrated *in vivo* in rat kidney (10) and rabbit skin (5). In our study the extent of vasodilation induced by amylin fragment was small, therefore the decrease in perfusion pressure did not reach statistical significance. Our results may be explained by the possibility that amylin fragment $_{8–37}$ may be less potent than amylin in inducing vasodilation.

In the present experiments the initial positive inotropic effect of CGRP is in agreement with the results of previous studies on isolated perfused guinea-pig hearts (9, 28) and rat ventricular myocytes (4). However, CGRP did not induce a positive contractile response in isolated perfused rat hearts (30). The doses of CGRP (26 pmol–1.3 nM) in this study were lower than those of our study (10 nM–1 μM) which might be responsible for the divergent contractile responses of CGRP. Manzini et al. (29) found that CGRP at the same doses applied in the present study caused a slight negative inotropic effect in guinea-pig hearts. Similar to this effect we have observed an insignificant negative inotropic effect of CGRP after the positive inotropy.

We have observed that CGRP causes positive chronotropic effect and a decrease in coronary perfusion pressure. Similar positive chronotropic and vasodilator effects of the peptide have been demonstrated in isolated guinea-pig and rat hearts, and also conscious and anaesthetized rats (11, 13, 16, 29, 30, 52).

It has been reported that the mean plasma concentrations of AM in rats and humans are $3.6\pm0.3\times10^{-9}$ and $3.3\pm0.4\times10^{-9}$ M, respectively (17, 46). In patients with acute myocardial infarction or congestive heart failure, plasma concentrations of AM increase several folds (22, 26). The clinical concentrations of the peptide are enough to

exert its effects on the heart. CGRP, at the concentration of 56 pM which is slightly above plasma concentration produces flush, hypotension, secondary catecholamine release and positive chronotropic effect in humans (42).

In conclusion, our results show that AM exerts negative inotropic, negative chronotropic and coronary vasodilatory effects on isolated rat hearts. Taking into account the effects of peptide and the elevated levels of plasma AM in patients with hypertension (25) myocardial infarction (2), heart failure (15) and septic shock (14), use of AM may be beneficial in these diseases. Amylin $_{8-37}$ induces biphasic contractile response and positive chronotropic effects. CGRP produces positive inotropic, positive chronotropic and vasodilator effects. CGRP may be useful in heart failure and myocardial infarction conditions, in which high levels of CGRP-like immunoreactivity were demonstrated (41).

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