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Adrenomedullin and myocardial contractility in the rat

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Abstract

The effects of adrenomedullin in the regulation of myocardial contractility were investigated in the rat. In papillary muscles ($n = 6$), adrenomedullin (0.1 to 10 nM) failed to show contractile effects. NO (nitric oxide) synthase inhibition with N^G-nitro-L-arginine (L-NOARG) did not unmask any inotropic effect of adrenomedullin. The positive inotropic effect of isoprenaline (0.01 nM to 10 μM) was identical after adrenomedullin, after L-NOARG, and after L-NOARG plus adrenomedullin ($n = 6$ each). In field-stimulated rat ventricular myocytes, adrenomedullin (1, 10, and 100 nM; $n = 4$ each) had impact neither on cell shortening nor on Ca^{2+} transients. In isolated constant-flow perfused hearts (7.3 ± 0.3 ml/min), adrenomedullin (1 nM, $n = 9$; 10 nM, $n = 7$) induced significant coronary vasodilation (−28%, −50%). In conclusion, adrenomedullin is a potent coronary vasodilator, but has no significant effects on myocardial contractility in the rat.

Keywords: Adrenomedullin; (Rat); Myocyte; Papillary muscle; Heart, isolated; Ca^{2+} transient

1. Introduction

Adrenomedullin, a recently identified potent endogenous vasodilator and natriuretic peptide, is expressed in a variety of cell types. Whereas the vasodilator properties appear to be well characterised (Kitamura et al., 1993; Charles et al., 1999), controversial reports exist up to now concerning the effects of this peptide on myocardial contractility. In 1993, Perret et al. suggested that bolus administration of adrenomedullin induces a mild and short-lived direct negative inotropic effect in the in vitro rat heart (Perret et al., 1993). A more recent study also described a direct negative inotropic effect of adrenomedullin in isolated rabbit cardiac ventricular myocytes. This decrease in myocyte contractility was due to a reduction in $[Ca^{2+}]_i$ and I_{ca} and appeared to be due to a nitric oxide (NO)-mediated increase of intracellular cGMP (Ikenouchi et al., 1997). In contrast to the previous publications, positive inotropic effects of adrenomedullin via cAMP-independent mechanisms (Szokodi et al., 1996, 1998) or through a cAMP-dependent pathway (Ihara et al., 2000) have also been reported.

In order to further elucidate the effect of adrenomedullin on myocardial contractility, we investigated the inotropic effects of this peptide by different experimental approaches using papillary muscles and isolated single adult cardiomyocytes. In addition to the contractile state, we determined changes in intracellular Ca^{2+} concentrations, evaluated by changes in Fluo-3 fluorescence in field stimulated cardiomyocytes.

2. Methods

2.1. Papillary muscle

Wistar rats of 180–200 g body weight were anaesthetised by intraperitoneal injection of pentobarbital sodium (50 mg/kg i.p.). Hearts were excised and subjected to retrograde perfusion with Krebs–Henseleit solution to remove blood elements. The Krebs–Henseleit solution consisted of (in mM) 116 NaCl, 25 $NaHCO_3$, 1.0 $CaCl_2$, 4.0 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 6 HEPES, and 10 glucose, and was equilibrated with 95% O_2/5% CO_2 (28°C, pH 7.4). After 5 min of perfusion, the buffer was changed to

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Krebs–Henseleit solution containing 20 mM butanedione monoxime to prevent the heart from beating during dissection of muscles. Long (slack length, 3.7 ± 0.5 mm) and thin (diameter, 0.37 ± 0.12 mm) preparations were cut along fibre directions from left ventricular papillary muscles. Preparations were fixed to a force transducer (Muscle Research System, Scientific Instruments, Heidelberg, Germany) and stimulated electrically via steel tweezers (biphasic rectangular pulse, 5 V, 5 ms). Experiments were conducted at 28°C in the Krebs–Henseleit buffer described above, in isometric contraction mode at a rate of 30/min. Preparations were allowed to equilibrate for at least 45 min at low preload (slack position); thereafter, they were carefully stretched over 20 min to the optimal length, l_{max}, at which the active developed force attained a maximum. Final preload was then adjusted to 50% of the passive force at l_{max} and, following another equilibration period of 30 min, preparations were randomly assigned to the following experimental groups.

2.1.1. Control ($n = 6$)

This group served to document time dependency under baseline conditions. Preparations were stimulated over 2 h; thereafter, dose–response curves for isoprenaline were performed to demonstrate preserved inotropic reserve. To this end, isoprenaline concentration in perfusion buffer was cumulatively increased every 5 min to 0.1, 1, 10, 100 nM, 1 μM, and finally to 10 μM.

2.1.2. Adrenomedullin ($n = 6$)

Adrenomedullin concentration in perfusion buffer was cumulatively increased every 40 min from 0.1 to 1, and to 10 nM. Dose–response curves for isoprenaline were then constructed as described above.

2.1.3. Adrenomedullin + L-NOARG ($n = 6$)

Since a recent study suggested that adrenomedullin has a negative inotropic effect mediated by NO (Ikenouchi et al., 1997), responses to adrenomedullin and isoprenaline were tested as described above, but in the presence of 100 μM L-NOARG to reveal any potential contribution of NO.

2.1.4. L-NOARG ($n = 6$)

Preparations were stimulated over 2 h in the presence of 100 μM L-NOARG. Dose–response curves for isoprenaline were then constructed.

2.1.5. Endothelin-1 ($n = 6$)

Endothelin-1 is known to evoke slowly developing positive inotropic changes (Li et al., 1991); endothelin-1 therefore served as positive control in our study. Endothelin-1 concentration in perfusion buffer was cumulatively increased every 40 min from 0.1 to 1, and to 10 nM.

The following parameters were documented: T_{dev}, peak developed tension; dT/dt_{max}, maximum rate of tension development; dT/dt_{min}, maximum rate of tension decline; time to peak tension; and RT_{50}, time from peak tension to 50% relaxation.

2.2. Adult field stimulated ventricular cardiomyocyte

Adult rat cardiac myocytes were isolated as recently described (Kubin et al., 1999). Briefly, hearts were perfused for 3 min with oxygenated Ca^{2+}-free Krebs–Henseleit buffer (37°C; pH 7.4) containing (in mM) 110 NaCl, 2.6 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 25 *N*-2-hydroxyethylpiperazine-*N*′-2-ethansulfonic acid (HEPES), and 11 glucose. After 30 min of collagenase digestion (30-μM Ca^{2+} and collagenase type II), the hearts were minced and incubated for another 15 min. The following washing steps increased $[Ca^{2+}]_o$ incrementally, from 200 and via 500 to 1000 μM. Cells were layered over a 4% bovine serum albumine gradient and turned down for 1 min at 19 g; the resulting pellet was then resuspended in experimental buffer (in mM): 117 NaCl, 2.8 KCl, 0.6 $MgCl_2$, 1.2 KH_2PO_4, 1.2 $CaCl_2$, 20 glucose, and 10 HEPES, pH 7.3. Typically, about 2×10^6 cells per rat heart were obtained, 95% of which showed the typical rod-shaped morphology with no blebs or granulations.

The cells were plated on four-well chamber glass slides (Nunc, Naperville, IL) which had been coated with 300 μl Laminin (10 μg/ml) for 1 h. After plating and a subsequent attachment period of 30 min, the buffer was exchanged for a staining solution containing 0.1% dimethyl sulphoxide (DMSO), 0.025% Pluronic F-127, 0.2% bovine serum albumine, and 5-μM Fluo 3-AM (F-6142: Sigma, Deisenhofen, Germany). Cells were incubated at room temperature for 45 min on an orbital shaker oscillating at 40 rpm. After incubation, the loading solution was replaced with fresh buffer and the incubation continued for an additional 30 min (in most cases, on the microscope stage).

The cells were incubated on a chamber slide with a volume of 500 μl experimental buffer. Bipolar electric pulses were generated by a custom-built stimulator and delivered to the cells via a pair of silver–chloride electrodes. The frequency was 1 Hz with an impulse duration of 5 ms. Cells were superperfused throughout the experiment with experimental buffer (23°C, 2 ml/min), with the use of a commercially available roller pump. The chamber slides were mounted onto the stage of a Nikon Diaphot TMD microscope (Nikon, Tokyo, Japan) equipped with a plan apochromate × 40 oil-immersion objective (NA 1.0). Ca^{2+}-dependent changes in Fluo-3 fluorescence (Ca^{2+} transients) were recorded with an Odyssey XL confocal laser scan microscope (Noran Instruments, Middleton, WI) using an argon ion laser (488-nm excitation light of 6 mW). Emitted light was long-pass filtered (> 515 nm) and collected at a frame rate of 120 images/s (626×114 pixel; pixel size 0.26×0.26 μm). No attempt was made to calibrate cytosolic Ca^{2+} because of the uncertain subcellu-

Table 1
Contractile parameters of papillary muscle preparations at baseline

	T_{dev} (mN/mm^2)	dT/dt_{max} (mN/mm^2/s)	dT/dt_{min} (mN/mm^2/s)	Time to peak tension (ms)	RT_{50} (ms)
Control	40 ± 2	866 ± 43	501 ± 25	75 ± 3	40 ± 2
Adrenomedullin	41 ± 3	853 ± 32	510 ± 20	74 ± 3	42 ± 3
L-NOARG	38 ± 2	826 ± 45	485 ± 29	75 ± 5	43 ± 3
Adrenomedullin + L-NOARG	39 ± 1	847 ± 50	490 ± 25	76 ± 4	41 ± 1
Endothelin-1	42 ± 3	856 ± 53	499 ± 21	75 ± 4	41 ± 2

Experiments were conducted at 28°C, in isometric contraction mode at a rate of 30/min. T_{dev}, peak developed tension; dT/dt_{max}, maximum rate of tension development; dT/dt_{min}, maximum rate of tension decline; RT_{50}, time from peak tension to 50% relaxation.

lar compartmentation (Spurgeon et al., 1990). Experiments were performed at room temperature (23°C) to minimise cell leakage of fluorescent probes (Spurgeon et al., 1990).

Data were stored on the hard disk of an Indy workstation (Silicon Graphics, USA). At an interval of every 8 ms, Ca^{2+} transients and cell length were determined using a custom-written macro function for Object-Image (Vischer et al., 1994).

The effects of different concentrations of adrenomedullin on the Ca^{2+} transients were evaluated from the changes in Fluo-3 fluorescence provided by the software as relative fluorescence units (rfu). The effects of adrenomedullin were ascertained once stable, steady-state conditions were reached. Each cardiomyocyte was derived from a separate animal, and was used for only a single investigation of one out of three different concentrations (1, 10, 100 nM, $n = 6$ each). To evaluate early and late effects of adrenomedullin, measurements were performed at 1, 3, 6 min and 10, 15, 20 min in the same cardiomyocyte. Changes in Ca^{2+} transients and cell shortening were expressed as percentage changes with reference to their respective baseline levels under control conditions.

Since endothelin-1 has been reported to increase cell shortening and Ca^{2+} transients in isolated rat cardiomyocytes (Katoh et al., 1998), we used this peptide (1 nM, $n = 3$) as positive control in our experimental setup.

2.3. Isolated heart preparation

The isolated perfused heart model was similar to that described previously (Felix et al., 1997). Male Wistar rats weighing 200 to 300 g were used for these experiments. After intraperitoneal anaesthesia (pentobarbital sodium 50 mg/kg body weight), the hearts were quickly excised and mounted for perfusion by the Langendorff technique. The hearts were perfused with a modified Krebs–Henseleit solution (in mM): NaCl 127.1, KCl 4.7, $MgSO_4$ 1.1, KH_2PO_4 1.19, $NaHCO_3$ 24.9, $CaCl_2$ 1.26, glucose 8.93, HEPES 10), equilibrated with 95% O_2/5% CO_2 (37°C, pH 7.4). Left ventricular pressures were recorded via a fluid-filled latex balloon inserted through the mitral valve and attached to a pressure transducer and chart recorder. The left ventricular end-diastolic pressures were maintained at 5 mm Hg. Balloon pressures were electronically differentiated to yield dP/dt and heart rate. Coronary flow rates were monitored with an ultrasonic flow meter (Transsonic) connected to a flow probe installed in the aortic arch. Coronary perfusion pressure was monitored with a pressure transducer attached to the aortic perfusion cannula. All parameters were continuously displayed. Registration included heart rate, left ventricular pressure, left ventricular peak dP/dt ($LVdP/dt_{max\ and\ min}$), coronary flow rates, and coronary perfusion pressure. After reaching steady-state conditions, adrenomedullin was infused in isolated hearts perfused at constant flow (7.3 ± 0.3 ml/min) into the aortic perfusion cannula in two different concentrations (1 nM, $n = 9$; 10 nM, $n = 7$) for at least 20 min.

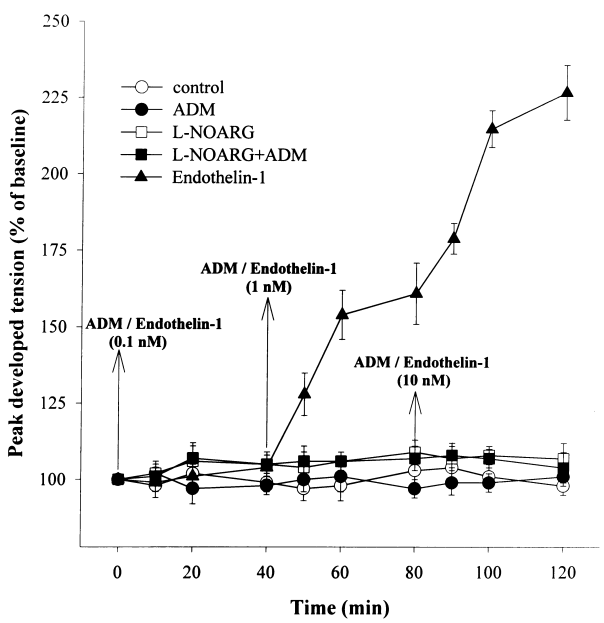

Fig. 1. Course of peak developed tension in papillary muscle preparations. Experiments were conducted at 28°C, in isometric contraction mode, at a rate of 30/min. Preparations served as time controls, or were stimulated in the presence of incrementing concentrations of rat adrenomedullin (ADM, 0.1, 1, and 10 nM), in the presence of the nitric oxide synthase inhibitor L-NOARG (100 μM), or in the presence of L-NOARG (100 μM) plus ADM (0.1, 1, 10 nM). Endothelin-1 (0.1–10 nM), known to evoke slowly developing positive inotropic effects, was used as positive control. Number of experiments was $n = 6$ in each case.

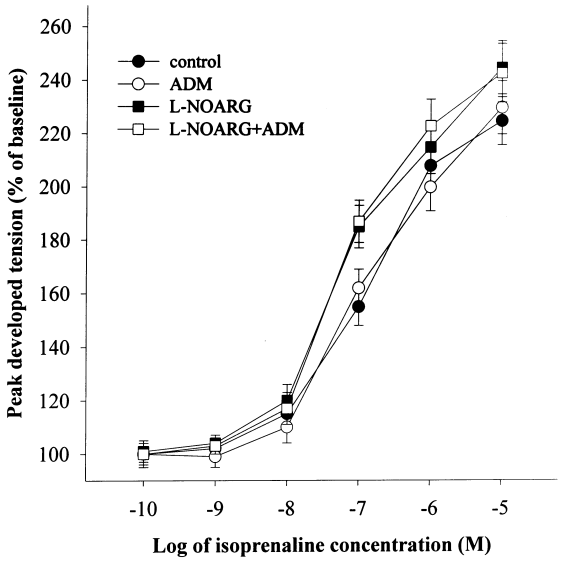

Fig. 2. Inotropic response to isoprenaline (0.1 nM–10 μM) as indicated by the course of peak developed tension in papillary muscle preparations. Experiments were conducted at 28°C, in isometric contraction mode at a rate of 30/min. Dose–response curves were constructed in time controls, in the presence of rat adrenomedullin (ADM, 10 nM), in the presence of the nitric oxide synthase inhibitor L-NOARG (100 μM), or in the presence of L-NOARG (100 μM) plus ADM (10 nM). Number of experiments was $n = 6$ in each case.

2.4. Statistical analysis

Results are expressed as mean values ± S.E.M. for n calculations. Effects of the indicated dilutions of adrenomedullin were evaluated by using non-parametric repeated measures analysis of variance with data alignment (Bortz et al., 1990). The analyses involved comparisons between groups (controls vs. adrenomedullin), and within groups. Uni-variate post-hoc analyses were performed (Mann–Whitney U-tests) after overall testing at a level of significance of $P < 0.05$. Accounts for multiple comparisons were carried out using the sequentially rejective Bonferroni–Holm procedure (Holm, 1979).

2.5. Drugs

Unless otherwise stated, reagents and substances were purchased from Sigma (Deisenhofen, Germany); rat adrenomedullin was obtained from Bachem (Heidelberg, Germany).

3. Results

3.1. Papillary muscle

As shown in Table 1, contractile parameters at baseline were nearly identical in all groups. Fig. 1 shows the course of peak developed tension in all experimental groups: application of adrenomedullin in incrementing concentrations, from 0.1 to 10 nM, changed none of the contractile parameters stated above. This finding was independent of the inhibition of NO synthase by L-NOARG (100 μM). In contrast, endothelin-1 at 1 and 10 nM significantly increased T_{dev} peak developed tension to 161 ± 10% and to 227 ± 9%, respectively. In addition, RT_{50} increased to

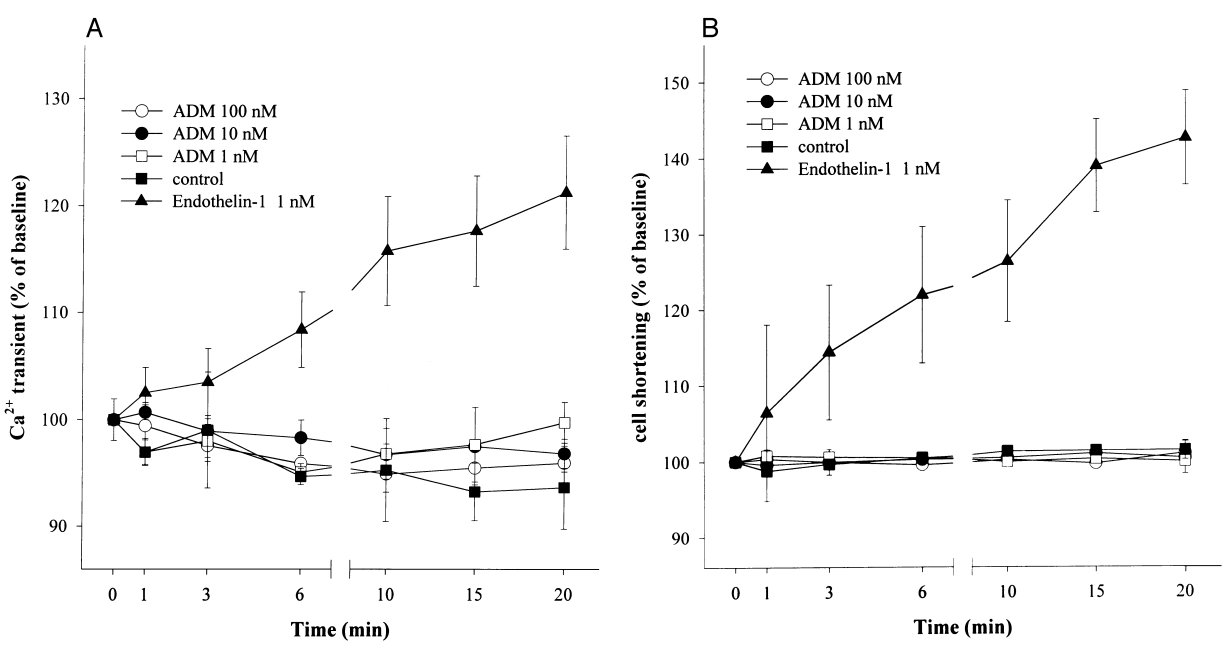

Fig. 3. Percentage changes in Ca^{2+} transient (Fluo-3 fluorescence), calculated as peak systolic relative fluorescence units minus diastolic relative fluorescence units (A), and cell length (B) of a single field-stimulated rat cardiomyocyte under basal conditions (0), and during superfusion with adrenomedullin (ADM, 100, 10, 1 nM). Number of experiments was $n = 6$ in each case. Endothelin-1 (1 nM) was used to document preserved inotropic response ($n = 3$).

Table 2
Coronary perfusion pressure, left ventricular contractile parameters, and heart rate in isolated hearts at baseline

	Coronary perfusion pressure (cm H_2O)	Left ventricular pressure (mm Hg)	LV$dP/dt_{max}$ (mm Hg/s)	Heart rate (beats/min)
Adrenomedullin (1 nM), $n = 9$	79 ± 3	80 ± 6	3294 ± 243	311 ± 10
Adrenomedullin (10 nM), $n = 7$	84 ± 2	78 ± 5	3149 ± 138	304 ± 9

Experiments were performed under constant-flow perfusion. LV$dP/dt_{max}$ = maximal left ventricular dP/dt.

135 ± 8% and 167 ± 7%; dT/dt_{max} to 165 ± 10% and 215 ± 11%, and dT/dt_{min} to 119 ± 4% and 153 ± 8%. Time to peak tension was significantly shortened, to 89 ± 2% of baseline, only by the highest endothelin-1 concentration used: 10 nM.

At the end of the experiments, preservation of inotropic response to isoprenaline was demonstrated by maximum increases in peak developed tension to 225 ± 9% (controls), 230 ± 10% (adrenomedullin), 245 ± 10% (L-NOARG), and 243 ± 11% (L-NOARG plus adrenomedullin) of baseline values (Fig. 2). There was no significant difference between maximum peak developed tension in controls and in the presence of L-NOARG. In addition, inotropic response to isoprenaline was not modulated by combined nitric oxide synthase inhibition and adrenomedullin administration.

3.2. Adult field stimulated ventricular cardiomyocyte

Fig. 3 shows the dependence of systolic cell shortening and Ca^{2+} transients on the concentration of adrenomedullin. At baseline, the rat ventricular myocytes shortened during stimulation by 7.3 ± 0.4% (mean ± S.E.M.), and Fluo-3 fluorescence increased from diastolic 23.1 ± 1.9 to peak systolic 59.1 ± 5.4 rfu. The effects of adrenomedullin were evaluated once stable, steady-state conditions were reached. Field-stimulated cardiomyocytes were superfused with adrenomedullin during a period of 20 min. Measurements of cell shortening and Ca^{2+} transients were performed at 1, 3, 6 and 10, 15 and 20 min in the same cardiomyocyte. Adrenomedullin tested in three different concentrations (1, 10, 100 nM) modified neither cell contractions nor Ca^{2+} transients ($n = 6$) (Fig. 3). Endothelin-1 (1 nM), used as positive control to document preserved inotropic response in this model, induced a time-dependent increase in cell shortening and Ca^{2+} transients (Fig. 3).

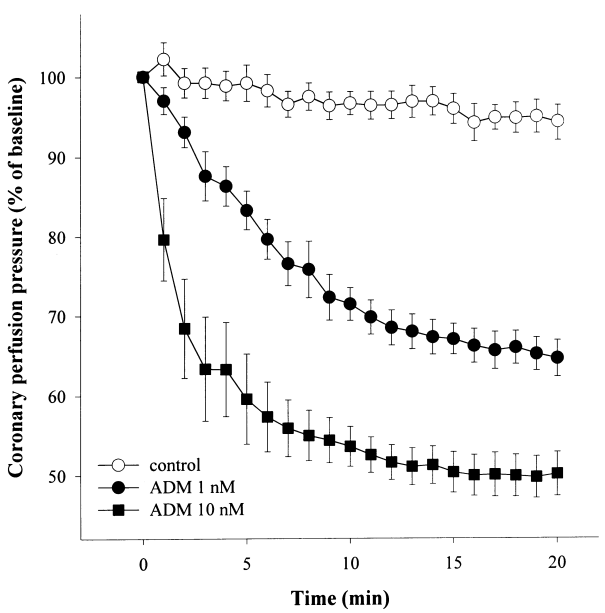

Fig. 4. Concentration/response curves of changes in coronary perfusion pressure in isolated rat hearts perfused at constant flow (7.3 ± 0.3 ml/min) in response to adrenomedullin (ADM, 1 nM, $n = 9$; 10 nM, $n = 7$). Values are means ± S.E.M.

3.3. Isolated Langendorff-perfused heart

Left ventricular contractile parameters, heart rate and coronary perfusion pressure were similar at baseline in both groups (Table 2). As depicted in Fig. 4, adrenomedullin infused intracoronarily into isolated hearts in two different concentrations (1 and 10 nM) induced a decrease in coronary perfusion pressure by 28% and 50%, respectively. This coronary vasodilation occurred within seconds of administration, and was maximal at 15 to 20 min. Heart rate remained stable. Under control conditions (without adrenomedullin infusion), coronary perfusion pressure did not change significantly during the experimental period (20 min) (Fig. 4). Vasodilation was paralleled by a decrease in left ventricular pressure, LV$dP/dt_{max\ and\ min}$ (data not shown). Since in the isolated heart perfused at constant flow, a decrease in coronary perfusion pressure is usually accompanied by a fall in left ventricular contractile parameters (Gregg phenomenon), no clear statement concerning the effects of adrenomedullin on myocardial contractility is allowed in this perfusion mode.

4. Discussion

The objective of the present study was to determine the effects of adrenomedullin on myocardial contractility. By using different in vitro assay systems, we clearly showed

that adrenomedullin failed to induce significant inotropic effects in the rat heart: in the rat papillary muscle, adrenomedullin did not influence twitch contractions and time constants. Furthermore, in accordance with the previous findings, simultaneous detection of cell shortening and Ca^{2+} transients in single ventricular rat cardiomyocytes by confocal microscopy likewise failed to show significant changes during adrenomedullin superfusion. Endothelin-1, which was used as positive control due to its proven slowly developing positive inotropic effects, induced an increase in twitch contraction in the papillary muscle, as well as cell shortening and Ca^{2+} transients in isolated cardiomyocytes. In the isolated Langendorff-perfused heart, dose-dependent coronary vasodilation was observed during adrenomedullin infusion, which verified the efficacy of the peptide.

In 1993, Perret et al. described in the isolated rat heart a mild negative inotropic effect of adrenomedullin after bolus administration, in addition to a marked vasodilating effect. This effect was rapid in onset, reached a peak at 60 s, and lasted up to 5 min (Perret et al., 1993). A negative inotropic effect of adrenomedullin was also found by Ikenouchi et al. in isolated adult rabbit cardiac ventricular myocytes accompanied by a decrease in $[Ca^{2+}]_i$ (Ikenouchi et al., 1997). The authors suggested a contribution of NO to the negative inotropic effect, since both an NO synthase inhibitor, N^G-monomethyl-L-arginine, blocked the negative inotropic effect of adrenomedullin, and the intracellular cGMP content increased in a concentration-dependent manner.

In contrast to the previous publications, several studies have reported that adrenomedullin has most likely positive inotropic effects. Thus, systemic administration of adrenomedullin in mammals resulted in an increase in cardiac output and stroke volume (Ishiyama et al., 1993, 1995; Parkes, 1995). However, in the in vivo-situation, it must be taken into account that this increase in cardiac output is primarily secondary to a fall in cardiac afterload as a result of decreasing mean arterial pressure. The present study was therefore not able to clarify whether the effects of adrenomedullin on cardiac function are secondary to changes in afterload, or whether they are mediated by direct actions on the heart. Findings by Szokodi et al. in the isolated perfused rat heart suggested that adrenomedullin exerts direct, positive inotropic action, via Ca^{2+} release from ryanodine and thapsigargin-sensitive Ca^{2+} stores, activation of protein kinase C, and Ca^{2+} influx through L-type Ca^{2+} channels, without increasing cAMP levels (Szokodi et al., 1998). Very recently, Ihara et al. reported that adrenomedullin has positive inotropic effects in papillary muscles, at least partially through a cAMP-dependent pathway (Ihara et al., 2000).

The underlying reasons for the discrepancies between the studies cited above and our findings are unclear. Methodological differences, in any case, are subject to discussion. Compared to Ihara et al., in particular, there is a difference in the composition of the perfusion buffers. The discrepancies in findings could stem from the fact that we worked with appreciable lower Ca^{2+} values (1 mM) in the perfusion buffer than did Ihara et al. (2.5 mM). Despite this lower extracellular Ca^{2+} concentration, however, we obtained initial values for peak developed tension at baseline which were approximately 30% greater than did his group (Ihara et al., 2000). Szokodi et al. pre-dilated the vasculature by decreasing the coronary perfusion rate of the isolated hearts from 7 to 5 ml/min to exclude secondary effects caused by the vasorelaxation of coronary arteries induced by adrenomedullin (Szokodi et al., 1998). In contrast to the studies of Szokodi et al., we did not observe any positive inotropic effects in experimental models in which the confounding influence of changes in coronary perfusion is absent, i.e., in strips of cardiac muscle perfused in vitro and in isolated cardiomyocytes.

To further elucidate the lack of contractile effects of adrenomedullin in our study, one may argue that potential positive inotropic effects of this peptide (as described by Szokodi) are outweighed by its negative inotropic properties mediated by NO, as suggested by Ikenouchi (Szokodi et al., 1998; Ikenouchi et al., 1997). To work around this question, we tested the effects of adrenomedullin under conditions of inhibited NO synthesis. This approach, however, did not unmask a positive inotropic effect. In contrast to findings reported by Balligand et al. (1993), Keaney et al. (1996), and Gyurko et al. (2000) obtained in myocytes, isolated hearts and in vivo, we were not able to detect a significant augmentation of the β-adrenergic response under conditions of inhibited NO synthesis in papillary muscles. Agonist-stimulated NO release (substance P, 0.5 μM, $n = 4$), however, significantly decreases peak developed tension at baseline ($-18 \pm 2\%$) and attenuates the maximum inotropic response to isoprenaline ($185 \pm 6\%$ vs. $225 \pm 9\%$ in controls) (data not shown). This finding demonstrates an intact endogenous NO system in our preparations.

Taken all together, the reasons for the differences between the studies concerning the effects of adrenomedullin on myocardial contractility remain unclear, however, species differences and distinct experimental models must be taken into account. In our study, we demonstrate that adrenomedullin has no inotropic effects in different models: first, in the papillary muscle preparation, in which the muscle is contracting under defined preload conditions, no changes in twitch contraction could be ascertained, even at the highest dose used; second, at the single cellular level, cell shortening in isolated, field-stimulated, adult rat ventricular cardiomyocytes was not affected. In accordance with these contractile measurements, there were no alterations in Ca^{2+} transients during adrenomedullin application, evaluated from changes in Fluo-3 fluorescence in the cardiomyocytes. In conclusion, our results do not provide any evidence for direct inotropic effects of adrenomedullin in the rat.

References

Balligand, J.L., Kelly, R.A., Marsden, P.A., Smith, T.W., Michel, T., 1993. Control of cardiac muscle cell function by an endogenous nitric oxide signalling system. Proc. Natl. Acad. Sci. U. S. A. 90, 347–351.

Bortz, J., Lienert, G.A., Boehnke, K., 1990. Verteilungsfreie Methoden in der Biostatistik. Springer Verlag, Berlin, Germany.

Charles, J.C., Lainchbury, J.G., Lewis, L.K., Rademaker, M.T., Richards, A.M., Yandle, T.G., Nicholls, M.G., 1999. The role of adrenomedullin. Am. J. Hypertens. 12, 166–173.

Felix, S.B., Stangl, V., Frank, T.M., Harms, C., Berndt, T., Kästner, R., Baumann, G., 1997. Release of a stable cardiodepressant mediator after myocardial ischaemia during reperfusion. Cardiovasc. Res. 35, 68–79.

Gyurko, R., Kuhlencordt, P., Fishman, M.C., Huang, P.L., 2000. Modulation of mouse cardiac function in vivo by eNOS and ANP. Am. J. Physiol. 278, H971–H981.

Holm, S., 1979. A simple sequentially rejective multiple test procedure. Scand. J. Stat. 6, 65–70.

Ihara, T., Ikeda, U., Tate, Y., Ishibashi, S., Shimada, K., 2000. Positive inotropic effects of adrenomedullin on rat papillary muscle. Eur. J. Pharmacol. 390, 167–172.

Ikenouchi, H., Kangawa, K., Matsuo, H., Hirata, Y., 1997. Negative inotropic effect of adrenomedullin in isolated adult rabbit cardiac ventricular myocytes. Circulation 95, 2318–2324.

Ishiyama, Y., Kitamura, K., Ichiki, Y., Nakamura, S., Kida, O., Kangawa, K., Eto, T., 1993. Hemodynamic effects of a novel hypotensive peptide, human adrenomedullin, in rats. Eur. J. Pharmacol. 241, 271–273.

Ishiyama, Y., Kitamura, K., Ichiki, Y., Sakata, J., Kida, O., Kangawa, K., Eto, T., 1995. Haemodynamic responses to rat adrenomedullin in anaesthetized spontaneously hypertensive rats. Clin. Exp. Pharmacol. Physiol. 22, 614–618.

Katoh, H., Terada, H., Iimuro, M., Sugiyama, S., Qing, K., Satoh, H., Hayashi, H., 1998. Heterogeneity and underlying mechanism for inotropic action of endothelin-1 in rat ventricular myocytes. Br. J. Pharmacol. 123, 1343–1350.

Keaney, J.F., Hare, J.M., Balligand, J.L., Loscalzo, J., Smith, T.W., Colucci, W.S., 1996. Inhibition of nitric oxide synthase augments myocardial contractile responses to beta-adrenergic stimulation. Am. J. Physiol. 271, H2646–H2652.

Kitamura, K., Kangawa, K., Kawamoto, M., Ichiki, Y., Nakamura, S., Matsuo, H., Eto, T., 1993. Adrenomedullin: a novel hypotensive peptide isolated from pheochromocytoma. Biochem. Biophys. Res. Commun. 192, 553–560.

Kubin, T., Ando, H., Scholz, D., Bramlage, P., Kostin, S., Van Veen, A., Heling, A., Hein, S., Fischer, S., Breier, A., Schaper, J., Schaper, W., 1999. Microvascular endothelial cells remodel cultured adult cardiomyocytes and increase their survival. Am. J. Physiol. 276, H2179–H2187.

Li, K., Stewart, D.J., Rouleau, J.L., 1991. Myocardial contractile actions of endothelin-1 in rat and rabbit papillary muscles. Role of endocardial endothelium. Circ. Res. 69, 301–312.

Parkes, D.G., 1995. Cardiovascular actions of adrenomedullin in conscious sheep. Am. J. Physiol. 268, H2574–H2578.

Perret, M., Broussard, H., Legros, T., Burns, A., Chang, J.K., Summer, W., Hyman, A., Lippton, H., 1993. The effect of adrenomedullin on the isolated heart. Life Sci. 53, 377–379.

Spurgeon, H.A., Stern, M.D., Baartz, G., Raffaeli, S., Hansford, R.G., Talo, A., Lakatta, E.G., Capogrossi, M.C., 1990. Simultaneous measurement of Ca^{2+}, contraction, and potential in cardiac myocytes. Am. J. Physiol. 258, H574–H586.

Szokodi, I., Kinnunen, P., Ruskoaho, H., 1996. Inotropic effect of adrenomedullin in the isolated perfused rat heart. Acta Physiol. Scand. 156, 151–152.

Szokodi, I., Kinnunen, P., Tavi, P., Weckström, M., Toth, M., Ruskoaho, H., 1998. Evidence for cAMP-independent mechanisms mediating the effects of adrenomedullin, a new inotropic peptide. Circulation 97, 1062–1070.

Vischer, N.O.E., Huls, P.G., Woldringh, C.L., 1994. Object-image: an interactive image analysis program using structured point collection. Binary 5, 160–166.