Peptides 30 (2009) 796–802

Contents lists available at ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides

Effects of adrenomedullin on systolic and diastolic myocardial function☆

Ana Patrícia Fontes-Sousa, Ana Luísa Pires, Catarina Santos Carneiro, Carmen Brás-Silva, Adelino F. Leite-Moreira *

Department of Physiology, Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal

ARTICLE INFO

Article history:
Received 12 October 2007
Received in revised form 11 December 2008
Accepted 11 December 2008
Available online 24 December 2008

Keywords:
Adrenomedullin
Inotropism
Lusitropism
Myocardial stiffness

ABSTRACT

Adrenomedullin (AM) effects were studied in rabbit papillary muscles by adding increasing concentrations (10^{-10} to 10^{-6} M) either alone or after pre-treatment with L-NNA, indomethacin, AM22–52 (AM receptor antagonist), CGRP(8–37) (CGRP receptors antagonist), KT5720 (PKA inhibitor), as well as after endocardial endothelium (EE) removal. Passive length–tension relations were constructed before and after a single concentration of AM (10^{-6} M). AM concentration-dependently induced negative inotropic and lusitropic effects, and increased resting muscle length (RL). At 10^{-6} M, AT, dT/dt_{max} and dT/dt_{min} decreased 20.9 ± 4.9%, 18.3 ± 7.3% and 16.7 ± 7.8%, respectively, and RL increased to 1.010 ± 0.004 L/L_{max}. Correcting RL to its initial value resulted in a 26.6 ± 6.4% decrease of resting tension, indicating decreased muscle stiffness, also patent in the down and rightward shift of the passive length–tension relation. The negative inotropic effect of AM was dependent on its receptor, CGRP receptor, PKA, the EE and NO, while the effects of AM on myocardial stiffness were abolished by EE damage and NO inhibition. This latter effect represents a novel mechanism of acute neurohumoral modulation of diastolic function, suggesting that AM is an important regulator of cardiac filling.

1. Introduction

Adrenomedullin (AM) is a peptide identified and isolated from human pheochromocytoma [27], and initially annotated as a vasodilator peptide. AM acts as a circulating hormone, which elicits various biological activities in a paracrine or autocrine manner.

Human AM (hAM) is a 52 amino acid peptide with structural homology to calcitonin gene-related peptide (CGRP) [27]. AM is produced in several tissues (kidney, lung, and heart) [28], and its production is upregulated by several factors such as oxidative stress, pro-inflammatory cytokines, angiotensin II, hypoxia, hyperglycemia, infusion of natriuretic peptide, and aldosterone, among other factors [1].

There is increasing experimental and clinical evidence in support of an important role of AM in the pathophysiology of a variety of cardiovascular diseases. In spite of its relatively low plasmatic levels [27], various clinical studies have shown that they correlate with the severity of diseases, such as heart failure (HF), acute myocardial infarction, and hypertension [23,24,36,40,42].

At the cardiovascular level, AM can be synthesized and secreted from various cells, including vascular endothelial cells, vascular smooth muscle cells, cardiomyocytes and fibroblasts [1,13]. Furthermore, AM and its receptors are expressed in the normal and failing myocardium [44,46].

In normal animals [47] and in an ovine model of pacing-induced HF [53], AM was shown to reduce peripheral resistance and to increase cardiac output. These data have led investigators to suggest that AM may be involved in the control of cardiac function and that AM is activated in HF to modulate the opposing effects of the vasoconstricting and sodium-retaining factors endothelin-1 (ET-1) and angiotensin II.

The direct myocardial effects of AM remain, however, largely unknown. With regard to contractility, positive [2,20,60], negative [19,22,35,38,52], and no significant [41,54,55,59] inotropic effects have been reported. On the other hand, its effects on the diastolic properties of the myocardium were not yet investigated. Recent evidences have shown that these properties and more specifically myocardial stiffness can be acutely modulated by nitric oxide (NO) [57], ET-1 [31], angiotensin II [32] and urotensin II [15].

Diastolic HF has emerged over the last two decades as a separate clinical entity. Approximately half of the patients presenting with symptoms of congestive HF exhibit a near normal left ventricular systolic function at rest, which is thought to be caused by a predominant abnormality in diastolic function [49]. Determinants of diastolic function include myocardial relaxation and passive properties of the ventricular wall, such as myocardial

☆ Presented in part at the American Heart Association Scientific Sessions conference, 2007, in Orlando, FL.

* Corresponding author at: Department of Physiology, Faculty of Medicine, Alameda Professor Hernâni Monteiro, 4200-319 Porto, Portugal.
Tel.: +351 225513644; fax: +351 225513646.
E-mail address: amoreira@med.up.pt (A.F. Leite-Moreira).

0196-9781/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.peptides.2008.12.011

stiffness, wall thickness and chamber geometry (size or volume). Other determinants include the structures surrounding the ventricle, the left atrium, pulmonary veins and mitral valve, and heart rate [30].

So, the present study was conducted to characterize the systolic and diastolic myocardial effects of AM and to clarify the intracellular pathways that underlie them.

2. Materials and methods

2.1. Animals and tissue preparation

This investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, Revised 1996).

2.2. Myocardial effects of adrenomedullin

2.2.1. Experimental preparation

Isometric and isotonic contractions were measured in papillary muscles isolated from the right ventricle of rabbits. Male New Zealand White rabbits (*Oryctolagus cuniculus*; 1.3–2.6 kg; $n = 38$) were anesthetized with intravenous sodium pentobarbital (25 mg kg^{-1}). A left thoracotomy was performed, and beating hearts were quickly excised and immersed in a modified Krebs–Ringer (KR) solution (composition in mM: 98 NaCl, 4.7 KCl, 2.4 $MgSO_4 \cdot 7H_2O$, 1.2 KH_2PO_4, 4.5 glucose, 1.8 $CaCl_2 \cdot 2H_2O$, 17 $NaHCO_3$, 15 sodium pyruvate, 5 sodium acetate, and 0.02 atenolol) at 35 °C with cardioplegic 2,3-butanedione monoxime (BDM; 3%) and 5% Newborn calf serum. Atenolol was used to prevent β-adrenergic-mediated effects. The solutions were in equilibrium with 95% O_2 and 5% CO_2, to obtain a pH between 7.38 and 7.42.

The time from thoracotomy to dissection was ~3 min. The right ventricle was opened and papillary muscles were isolated by first dividing the chordae tendinae at the muscle tip and then freeing the muscle base and a small amount of surrounding myocardium from the ventricular wall. Only long, thin, uniformly cylindrical muscles were used.

After dissection, papillary muscles ($n = 62$; length: 4.9 ± 0.2 mm; weight: 3.9 ± 0.2 mg; preload: 3.4 ± 0.1 mN) were mounted vertically in a 10-ml plexi glass organ bath containing the aforementioned KR solution at 35 °C. The lower muscular end was fixed in a phosphorbronze clip, and the upper tendinous end was attached to an electromagnetic length–tension transducer (University of Antwerp, Belgium).

Preload was initially set between 3 and 4 mN according to muscle dimensions. The preparations were stimulated at 0.6 Hz with a voltage of 10% above threshold (typically 30–60 mV) by rectangular pulses of 5 ms duration through two platinum electrodes arranged longitudinally alongside the entire muscle. After 20 min later, bathing solutions were replaced by corresponding KR solutions without BDM and the muscle started to contract. One hour later, bathing solution was replaced by corresponding serum-free KR solution. During the next 2 h, the muscles were stabilized. Finally, the muscles were stretched to a muscle length at which active force development was maximal. At this point, this length (mm) known as maximum physiological length (L_{max}) was measured with a microruler. During the experiment, changes in diastolic muscle length and muscle shortening were measured by the isotonic transducer. Protocols were initiated after obtaining two similar isotonic and isometric control twitches separated by a 10 min interval.

At the end of the experiment the muscles were removed, lightly blotted and then weighed. Muscle cross-sectional area was calculated by dividing the weight of the muscle by its length at L_{max}. A cylindrical shape and a specific gravity of 1.0 were assumed [15]. Muscle tension was then expressed as force normalized per cross-sectional area (mN mm^{-2}).

2.2.2. Experimental protocols

Effects of increasing concentrations of human AM-(1–52) ($C_{264}H_{406}N_{80}O_{77}S_3$) (AM; 10^{-10} to 10^{-6} M) on contraction, relaxation, and diastolic properties of the myocardium were studied in rabbit papillary muscles in the following conditions: (A) control muscles with intact endocardial endothelium (EE), (B) after selective removal of EE by a brief (1 s) immersion of the papillary muscle in a weak solution (0.5%) of the detergent Triton X-100 [6,7], followed by abundant wash with Triton-free KR solution, and (C) in muscles with intact EE in the presence of: (i) N^G-nitro-L-arginine (L-NNA; 10^{-5} M), a NO synthase inhibitor; (ii) indomethacin (Indo; 10^{-5} M), a cyclooxygenase inhibitor; (iii) human AM-(22–52) ($C_{159}H_{252}N_{46}O_{48}$) (AM22–52; 10^{-6} M), an antagonist of AM receptor; (iv) α-CGRP(8–37) ($C_{139}H_{230}N_{44}O_{38}$) (CGRP(8–37); 10^{-6} M), a selective antagonist for CGRP receptors; (v) KT5720 (KT, 10^{-6} M), an inhibitor of PKA. These substances were dissolved in the KR solution before the addition of AM, and muscle twitches were recorded after a stable response was obtained, typically 15–20 min later. After that, AM was added cumulatively without any washout between. Finally, in another subset of muscles, passive length–tension relations were constructed in the absence and in the presence of the highest concentration of AM. Of note, that in each experimental protocol, all papillary muscles were obtained from different animals.

2.2.3. Data acquisition and analysis

Isotonic and isometric twitches were recorded and analyzed with dedicated software (University of Antwerp, Belgium). Selected parameters included: resting tension (RT; mN mm^{-2}), active tension (AT; mN mm^{-2}); maximal velocities of tension rise (dT/dt_{max}; mN mm^{-2} s^{-1}) and decline (dT/dt_{min}; mN mm^{-2} s^{-1}); peak isotonic shortening (PS; $\%L_{max}$); maximal velocities of shortening (dL/dt_{max}; L_{max} s^{-1}) and lengthening (dL/dt_{min}; L_{max} s^{-1}); time for half-relaxation (tHR, ms); and time to active tension (tAT; ms).

In the various protocols, results are given as percent change from baseline. For the parameters that are expressed as negative values (e.g. dT/dt_{min}) such percent change refers to the absolute values. When a pharmacological inhibitor was used or the EE damaged, the term baseline refers to the performance in the presence of those inhibitors or after damage of EE, before the addition of AM.

2.3. Drugs and materials

Drugs were obtained from the following sources: human AM-(1–52), human AM-(22–52) and human α-CGRP(8–37): Bachem (Bubendorf, Switzerland); all other chemicals: Sigma Chemical Co. (St. Louis, MO, USA). Stock solutions of all chemicals were dissolved in distilled water and stored at −20 °C until use.

2.4. Statistical methods

Values are presented as means ± standard error of mean (S.E.M.) and n represents the number of experiments. Effects of increasing concentrations of AM alone on the different experimental parameters were analyzed by one-way repeated-measures ANOVA. Effects of increasing concentrations of AM under various experimental conditions were analyzed with a repeated-measures two-way ANOVA. Effects on the various parameters of a single concentration of the antagonists were analyzed with a paired t-test. When significant differences were detected with any of the ANOVA tests, the Student–Newman–Keuls test was selected to perform pairwise multiple comparisons. $P < 0.05$ was accepted as significant.

Table 1
Morphologic and contractile characterization of papillary muscles ($n = 62$).

Parameter	Value
Length (mm)	4.9 ± 0.2
Weight (mg)	3.9 ± 0.2
Preload (mN)	3.4 ± 0.1
AT (mN/mm^2)	22.1 ± 1.4
dT/dt_{max} (mN/(mm^2 s))	142.8 ± 10.3
dT/dt_{min} (mN/(mm^2 s))	−120.7 ± 7.5
tHR (ms)	409.3 ± 9.4
tAT (ms)	260.0 ± 6.2

AT: active tension; dT/dt_{max}, dT/dt_{min}: maximum velocity of tension rise and decline, respectively; tHR: time for half-relaxation; tAT: time to active tension. Values are means ± S.E.M.

3. Results

Morphometric characteristics and baseline performance of papillary muscles did not vary significantly between the different experimental groups (means ± S.E.M. presented in Table 1). Concentration–response curves to AM in the various experimental conditions are illustrated in Figs. 1–6.

Effects of increasing concentrations of AM on myocardial contractility (inotropy) and relaxation (lusitropy) are illustrated in Fig. 1, where it can be seen that these concentrations decreased both contractility (AT and dT/dt_{max}) and lusitropy (dT/dt_{min}). The highest concentration of AM (10^{-6} M) decreased 20.9 ± 4.9% AT, 18.3 ± 7.3% dT/dt_{max}, 16.7 ± 7.8% dT/dt_{min}, 11.9 ± 3.8% PS, 13.7 ± 4.8% dl/dt_{max}, 10.9 ± 5.3% dl/dt_{min} ($P < 0.05$). Effects on tHR and tAT (onset of relaxation) were not statistically significant.

With regard to the diastolic properties of the myocardium, we observed that AM progressively increased resting muscle length (Fig. 2) at a constant resting tension. Correcting muscle length, at the end of the experiment, to its initial value resulted in a 26.6 ± 6.4% decrease of resting tension, without altering the other contractile parameters. These results indicate an increase in muscle distensibility, or on the other hand, a decrease in muscle stiffness. This aspect is further explored in Fig. 3 where passive length–tension relations at baseline and in the presence of AM (10^{-6} M) are depicted. In this figure, it can be seen that this relation is right and downward shifted by AM. In other words, at each resting tension, muscle length was always significantly greater in the presence of AM, indicating that this peptide acutely increases distensibility and lowers stiffness of the myocardium.

Effects of AM after damaging the EE, in the presence of a selective AM receptor antagonist (AM22–52) or a selective CGRP receptors antagonist (CGRP(8–37)), or after inhibition of cyclooxygenase (Indo), NO synthase (L-NNA), or PKA (KT) are illustrated in Figs. 4–6. While AM22–52, CGRP(8–37), Indo and KT did not significantly modify *per se* any of the analyzed contractile parameters, selective destruction of the EE or the presence of L-NNA resulted in a significant decrease of AT by 33.1 ± 5.6% and

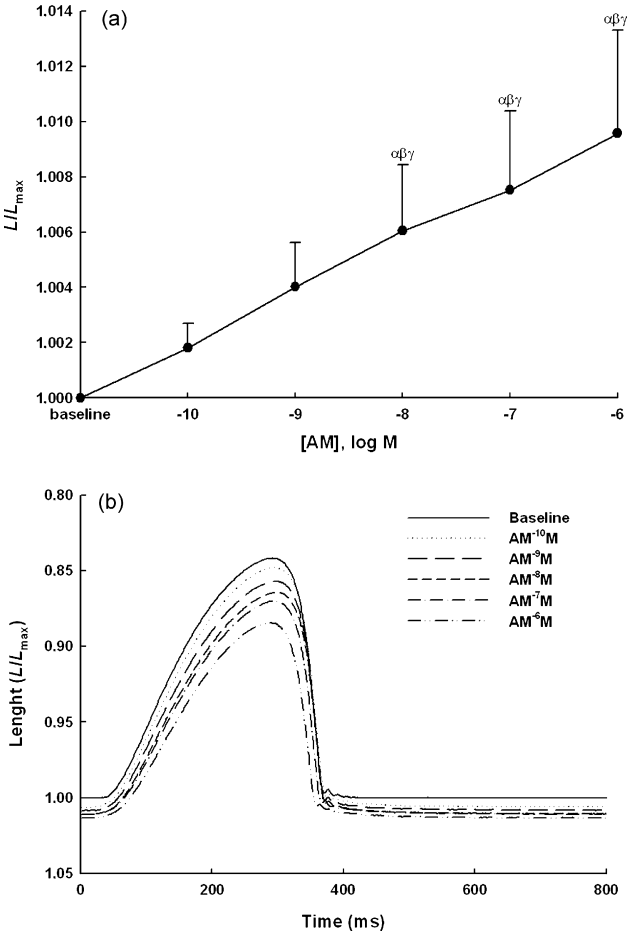

Fig. 2. Effect of increasing concentrations of adrenomedullin (AM, 10^{-10} to 10^{-6} M, $n = 9$) on (a) resting muscle length (L/L_{max}). Data are mean ± S.E.M., expressed as percent variation from baseline. $P < 0.05$: α vs. baseline, β vs. 10^{-10} M AM, γ vs. 10^{-9} M AM, δ vs. 10^{-8} M AM, ε vs. 10^{-7} M AM. Panel (b) shows a representative example of isotonic twitches at baseline and in the presence of increasing concentrations of AM.

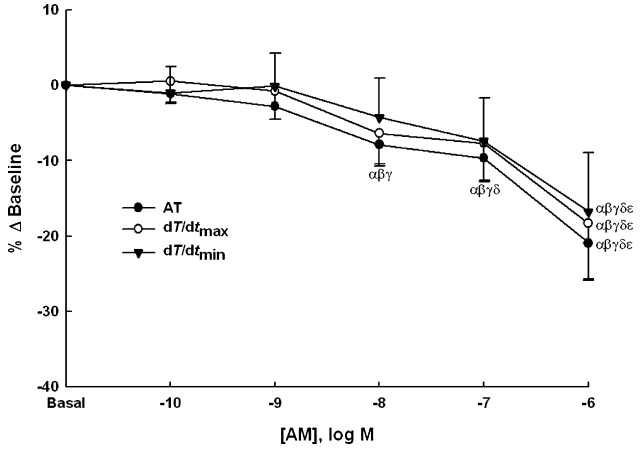

Fig. 1. Effect of increasing concentrations of adrenomedullin (AM, 10^{-10} to 10^{-6} M, $n = 9$) on active tension (AT) and peak rates of tension rise and decline (dT/dt_{max} and dT/dt_{min}, respectively). $P < 0.05$: α vs. baseline, β vs. 10^{-10} M AM, γ vs. 10^{-9} M AM, δ vs. 10^{-8} M AM, ε vs. 10^{-7} M AM.

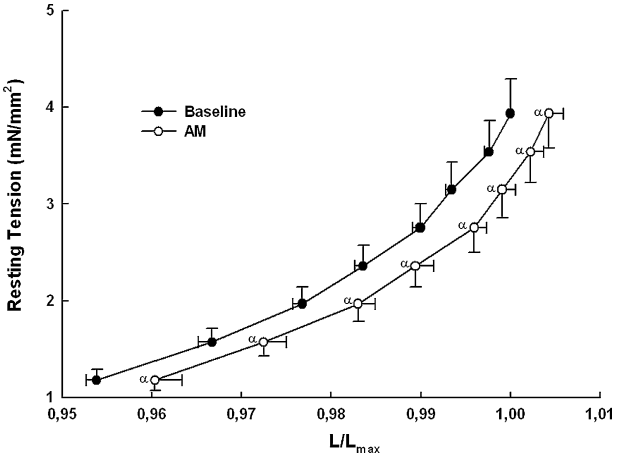

Fig. 3. Passive length–tension relations at baseline and in the presence of adrenomedullin (AM, 10^{-6} M, $n = 6$). Data are mean ± S.E.M. $P < 0.05$: α vs. baseline.

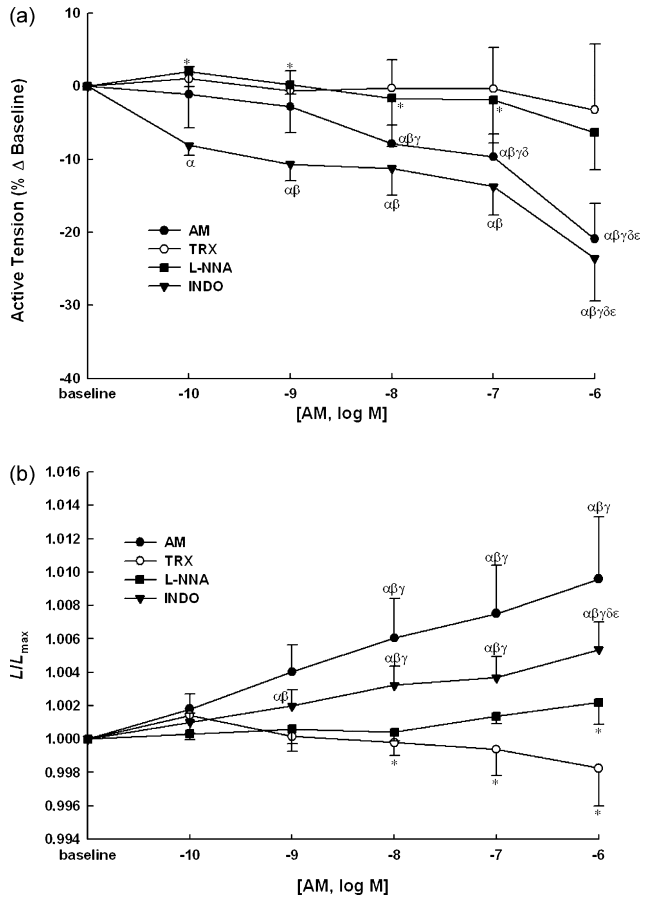

Fig. 4. Effect of increasing concentrations of adrenomedullin (AM, 10^{-10} to 10^{-6} M) on a active tension and b passive muscle length (L/L_{max}) in the absence ($n = 9$) or presence of damaged endocardial endothelium (TRX, $n = 9$), NO synthase inhibition (L-NNA, 10^{-5} M, $n = 7$), cyclooxygenase inhibition (INDO, 10^{-5} M, $n = 9$). Data are mean ± S.E.M., expressed as percent variation from baseline. $P < 0.05$: α vs. baseline, β vs. 10^{-10} M AM, γ vs. 10^{-9} M AM, δ vs. 10^{-8} M AM, ε vs. 10^{-7} M AM, * vs. AM alone.

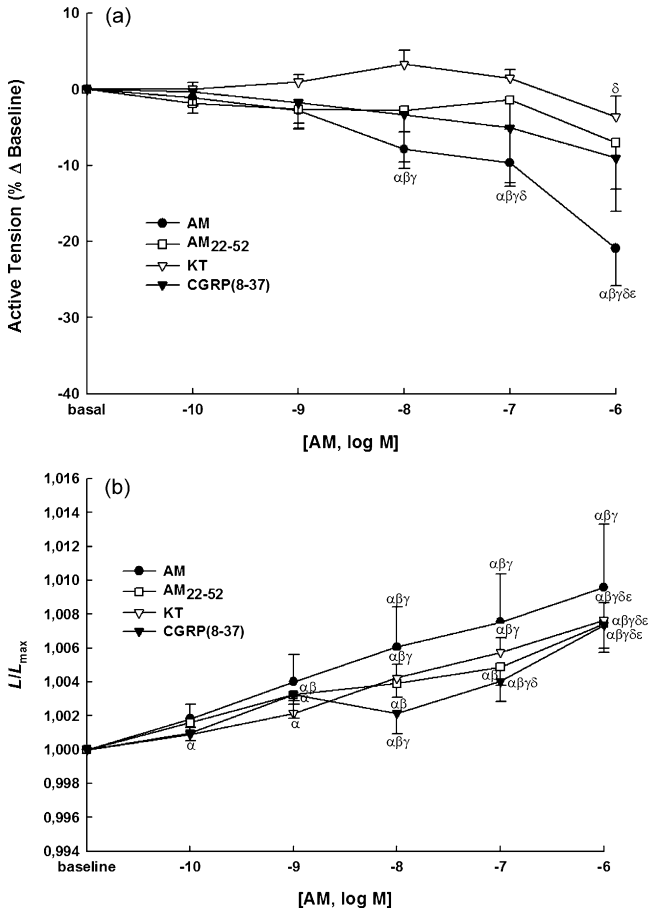

Fig. 5. Effect of increasing concentrations of adrenomedullin (AM, 10^{-10} to 10^{-6} M) on (a) active tension and (b) passive muscle length (L/L_{max}) in the absence ($n = 9$) or presence of selective AM receptor antagonist (human AM-(22–52)) (AM22–52, 10^{-6} M, $n = 8$), PKA inhibitor (KT5720) (KT, 10^{-6} M, $n = 7$) or selective CGRP receptors antagonist (α-CGRP(8–37)) (CGRP(8–37), 10^{-6} M, $n = 7$). Data are mean ± S.E.M., expressed as percent variation from baseline. $P < 0.05$: α vs. baseline, β vs. 10^{-10} M AM, γ vs. 10^{-9} M AM, δ vs. 10^{-8} M AM, ε vs. 10^{-7} M AM, * vs. AM alone.

5.8 ± 2.4%, dT/dt_{max} by 31.5 ± 6.4 and 4.6 ± 3.1% and dT/dt_{min} by 27.0 ± 6.8 and 6.5 ± 3.4%, respectively.

The myocardial effects of AM were also significantly altered by these agents. For instance, the negative inotropic effect of AM was abolished when the EE was damaged or in the presence of L-NNA (Fig. 4a). Furthermore, in the latter condition the effects of AM on passive muscle length were no more statistically significant, having been totally abolished when the EE was damaged (Figs. 4b and 6). On the other hand, AM22–52, CGRP(8–37) and KT blunted the negative inotropic effect of AM (Fig. 5a), but did not alter the effect of AM on resting length and tension (Figs. 5b and 6). Finally, none of the effects of AM were altered by Indo (Figs. 4a and b and 6).

4. Discussion

The present study shows that AM induces significant concentration-dependent negative inotropic and lusitropic effects, and an acute increase in myocardial distensibility. The former effects are completely abolished by AM or CGRP receptors blockade, PKA inhibition, EE removal or NO synthase inhibition. In contrast, the effect of AM on myocardial distensibility was no more observed when the EE was damaged or NO synthase inhibited. These observations suggest that this novel effect of AM requires an intact EE and is dependent of NO release.

A negative inotropic effect of AM was previously found in vitro [2,22,38,52], which is in line with our results. Nevertheless, this effect is apparently in disagreement with data from other in vitro studies [2,20,60], and from some in vivo studies [39,45] in which acute AM infusion increased cardiac index and stroke volume index. In vivo, this increase in cardiac output has been primarily attributed to a fall in cardiac afterload as a result of decreasing mean arterial pressure. A lack of inotropic and lusitropic effects of AM has also been reported in normal and heart-failure dogs [29]. Reasons for these discrepancies between studies presumably include species differences and distinct experimental models.

Though the major signal transduction pathway activated by AM appears to be Gs-mediated adenylate cyclase/cAMP/PKA system [20], not all effects of AM can be explained by this pathway [18]. A previous study suggested a contribution of NO to the negative inotropic effect promoted by AM in adult rabbit cardiac ventricular myocytes, which decreased intracellular Ca^{2+} concentration through a cGMP-dependent mechanism [22]. In the present study, besides NO and PKA, the negative inotropic effect of AM was also modulated both by its receptor, CGRP receptor and by the EE. Although the activation of the adenylate cyclase–cAMP system is one of the major pathways for the stimulation of cardiac contractility in the mammalian hearts [37], a recently published study observed a switch from G_s coupling to PKA-dependent G_i coupling with AM. This resulted in a shift from positive inotropy to negative inotropy, which was time dependent and dose dependent [34] and is consonant with our results.

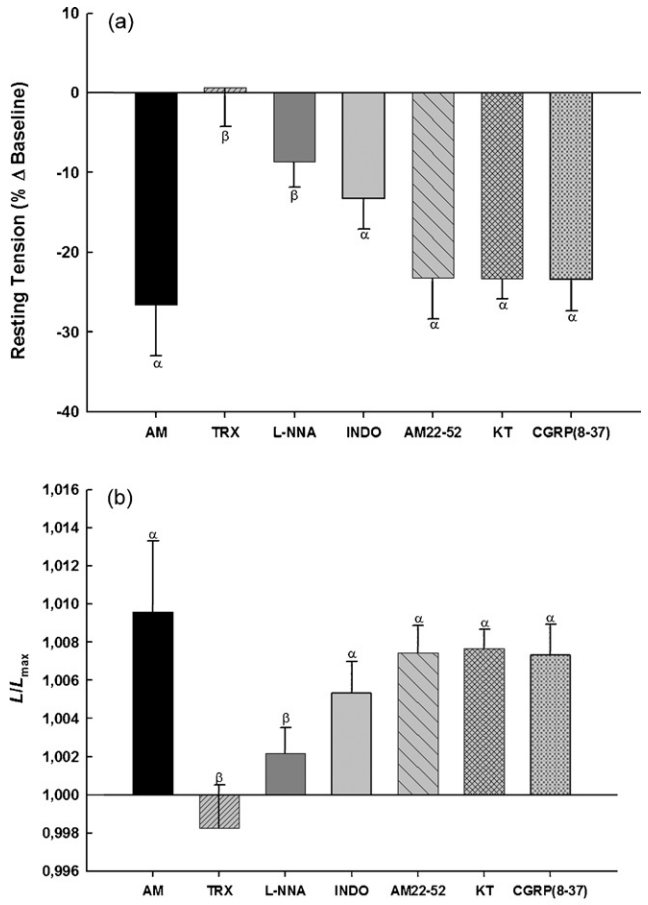

Fig. 6. Effects of adrenomedullin (AM, 10^{-6} M) on (a) resting tension and (b) resting muscle length (L/L_{max}) in the absence ($n = 9$) or presence of damaged endocardial endothelium (TRX, $n = 9$), NO synthase inhibition (L-NNA, 10^{-5} M, $n = 7$), cyclooxygenase inhibition (INDO, 10^{-5} M, $n = 9$), selective AM receptor antagonist (human AM-(22–52)) (AM22–52, 10^{-6} M, $n = 8$), PKA inhibitor KT5720 (KT, 10^{-6} M, $n = 7$) or selective CGRP receptors antagonist (α-CGRP(8–37)) (CGRP(8–37), 10^{-6} M, $n = 7$). Data are means ± S.E.M., expressed as percent variation from baseline. $P < 0.05$: α vs. baseline, β vs. AM alone.

Likewise other neurohumoral agents, such as NO [50], ET-1 [31], angiotensin II [32] and urotensin II [15], we observed that AM acutely modulates myocardial stiffness, which is an important determinant of ventricular filling and, therefore, of diastolic function [30]. This effect was significantly blunted by EE removal and by inhibition of NO. The EE has also been involved in the effect on distensibility of some of these neurohumoral agents [5,9,51]. Similarly to vascular endothelial dysfunction [11], it seems that cardiac endothelial dysfunction is present and/or may contribute to HF progression [4]. So, considering that cardiac endothelium, both vascular and endocardial, regulates performance of underlying cardiac muscle, the results of the present study could help to better understand the pathophysiology of HF.

Given that NO is one of the most important endothelial mediators and that many of the actions of AM occur via AM stimulated synthesis of NO, we investigated how this agent modulates the myocardial effects of this peptide. In vivo studies in rat [14], sheep [10], and humans [61] have suggested that AM-induced vasodilatation is mediated in part by stimulation of NO release from endothelial cells. This idea has been confirmed by in vitro studies, which showed that AM increases endothelial NO synthase activity by elevating intracellular-free calcium concentration ($[Ca^{2+}]_i$) [3,58] or by activating phosphatidylinositol 3-kinase and protein kinase B/Akt [43]. In cardiac myocytes, AM augments NO production via a cAMP-dependent signaling pathway [21], and, as cited earlier, NO mediates an AM-induced decrease in contractility of isolated myocytes [22].

In the current study, we found that after blocking NO release, AM-induced increase in resting muscle length (enhanced myocardial distensibility) was no more observed. In fact, it has been previously suggested that NO has an important role not only in the regulation of cardiac contractility [26], but also in the increase of diastolic distensibility [48,50]. Although our findings suggest that AM stimulates NO release from endocardial endothelium cells, in the present study we did not provide direct evidence of this fact, which represents a limitation of the study.

Specific AM receptors coupled to stimulation of adenylyl cyclase have been reported in myocardial tissue [25]. In addition, there is evidence for receptor sites that bind both AM and CGRP with fairly high affinity [63]. It was recently shown that the calcitonin receptor-like receptor (CRLR) can function either as an AM receptor or as a CGRP receptor, depending on the expression of different members of a novel family of single-transmembrane-domain proteins called receptor-activity-modifying proteins (RAMPs) [33,62]. So far, the RAMP family has been shown to consist of three isoforms: RAMP1, RAMP2 and RAMP3 [17,33,56]. Thus, the combination of CRLR plus RAMP2 results in an AM receptor 1 (AM_1), whereas CRLR co-expression with RAMP3 results in an AM receptor 2 (AM_2) [8,16].

In the present study, AM-induced negative inotropic effect was effectively blocked by both CGRP(8–37) and AM22–52, which suggests that AM may regulate cardiac function mediated by CGRP receptors and AM specific receptors in cardiac tissue. On the other hand, AM was observed to promote an increase of myocardial distensibility, through the activation of CGRP(8–37) or AM22–52 insensitive receptors. Although the AM peptide fragment AM22–52 has been described as an antagonist of both AM_1 and AM_2 receptors [12], a recent study demonstrated that AM22–52 is a more selective antagonist at the AM_1 (CRLR/RAMP2) than at the AM_2 (CRLR/RAMP3) receptor [17]. So, we hypothesize that the increase in myocardial distensibility induced by AM is possibly modulated by the AM_2 rather than by the AM_1 receptor, although further studies are needed to clarify these issues.

Finally, concerning the pathophysiologic relevance of our findings, we must point out that a decrease of 27% in passive tension of the isolated muscle indicate that AM might allow the ventricle to reach the same diastolic volume with almost 30% lower filling pressures, which is undoubtedly a potentially important adaptation mechanism. As the acute effects of AM on diastolic function were determined in an in vitro model, it allows determining the effects of AM on intrinsic myocardial diastolic properties, excluding those resulting from load and coronary tonus changes. However, the effects of AM in vivo, where other important adaptation mechanisms also affect diastolic filling pressures, may differ from those reported here.

On the other hand, the results of the present study emphasize that humoral influences on diastolic cardiac function are modulated by the interaction with EE and its mediators, such as NO, which being altered in the failing heart might provide new elements for the comprehension of the pathophysiology of HF.

5. Conclusions

Since its discovery, there has been great interest in AM as a promising endogenous peptide for the treatment of cardiovascular diseases. The present study provided new insights into the direct cardiac actions of AM. It described, for the first time, the modulation of diastolic function by AM, which represents a potentially powerful regulator of cardiac filling. These findings might improve our understanding about the role of AM, namely on

diastolic function, which has been greatly overlooked in most studies.

Acknowledgments

We thank Marta Oliveira for her expert technical assistance.

The collaboration of Dr. V. Monteiro-Cardoso and Dr. N. Pereira-Gonçalves in some experiments is also acknowledged.

Supported by the Portuguese Foundation for Science and Technology (grant no. POCI/SAU-FCT/60803/2004, partially funded by FEDER) through the Cardiovascular R&D Unit (FCT no. 51/94).

Ana Patrícia Fontes-Sousa and Ana Luísa Pires were supported by grants from the Portuguese Foundation for Science and Technology (nos. SFRH/BD/22590/2005 and SFRH/BD/19544/2004, respectively).

References

[1] Beltowski J, Jamroz A. Adrenomedullin—what do we know 10 years since its discovery? Pol J Pharmacol 2004;56:5–27.
[2] Bisping E, Tenderich G, Barckhausen P, Stumme B, Bruns S, von Lewinski D, et al. Atrial myocardium is the predominant inotropic target of adrenomedullin in the human heart. Am J Physiol 2007;293:H3001–7.
[3] Boussery K, Delaey C, Van de Voorde J. Influence of adrenomedullin on tone of isolated bovine retinal arteries. Invest Ophthalmol Vis Sci 2004;45:552–9.
[4] Bras-Silva C, Fontes-Sousa AP, Moura C, Areias JC, Leite-Moreira AF. Impaired response to ET(B) receptor stimulation in heart failure: functional evidence of endocardial endothelial dysfunction? Exp Biol Med (Maywood NJ) 2006;231:893–8.
[5] Bras-Silva C, Leite-Moreira AF. Obligatory role of the endocardial endothelium in the increase of myocardial distensibility induced by endothelin-1. Exp Biol Med (Maywood NJ) 2006;231:876–81.
[6] Brutsaert DL, De Keulenaer GW, Fransen P, Mohan P, Kaluza GL, Andries LJ, et al. The cardiac endothelium: functional morphology, development, and physiology. Prog Cardiovasc Dis 1996;39:239–62.
[7] Brutsaert DL, Meulemans AL, Sipido KR, Sys SU. Effects of damaging the endocardial surface on the mechanical performance of isolated cardiac muscle. Circ Res 1988;62:358–66.
[8] Buhlmann N, Leuthauser K, Muff R, Fischer JA, Born W. A receptor activity modifying protein (RAMP)2-dependent adrenomedullin receptor is a calcitonin gene-related peptide receptor when coexpressed with human RAMP1. Endocrinology 1999;140:2883–90.
[9] Castro-Chaves P, Pimentel-Nunes P, Fontes-Carvalho R, Soares JB, Martins J, Leite-Moreira AF. Angiotensin II-induced increase in myocardial distensibility is modulated by the endocardial endothelium, endothelin-1 and nitric oxide. J Am Coll Cardiol 2007;49(53A-A Suppl.).
[10] De Matteo R, May CN. Direct coronary vasodilator action of adrenomedullin is mediated by nitric oxide. Brit J Pharmacol 2003;140:1414–20.
[11] Drexler H, Hayoz D, Munzel T, Hornig B, Just H, Brunner HR, et al. Endothelial function in chronic congestive heart failure. Am J Cardiol 1992;69:1596–601.
[12] Eguchi S, Hirata Y, Iwasaki H, Sato K, Watanabe TX, Inui T, et al. Structure–activity relationship of adrenomedullin, a novel vasodilatory peptide, in cultured rat vascular smooth muscle cells. Endocrinology 1994;135:2454–8.
[13] Eto T, Kato J, Kitamura K. Regulation of production and secretion of adrenomedullin in the cardiovascular system. Regul Peptides 2003;112:61–9.
[14] Feng CJ, Kang B, Kaye AD, Kadowitz PJ, Nossaman BD. L-NAME modulates responses to adrenomedullin in the hindquarters vascular bed of the rat. Life Sci 1994;55:PL433–8.
[15] Fontes-Sousa AP, Brás-Silva C, Pires AL, Monteiro-Sousa D, Leite-Moreira AF. Urotensin II acutely increases myocardial length and distensibility: potential implications for diastolic function and ventricular remodeling. Naunyn Schmiedebergs Arch Pharmacol 2007;376(1–2):107–15.
[16] Hay DL, Conner AC, Howitt SG, Smith DM, Poyner DR. The pharmacology of adrenomedullin receptors and their relationship to CGRP receptors. J Mol Neurosci 2004;22:105–13.
[17] Hay DL, Howitt SG, Conner AC, Schindler M, Smith DM, Poyner DR. CL/RAMP2 and CL/RAMP3 produce pharmacologically distinct adrenomedullin receptors: a comparison of effects of adrenomedullin 22–52, CGRP 8–37 and BIBN4096BS. Brit J Pharmacol 2003;140:477–86.
[18] Hay DL, Smith DM. Adrenomedullin receptors: molecular identity and function. Peptides 2001;22:1753–63.
[19] Hyvelin JM, Shan Q, Bourreau JP. Adrenomedullin: a cardiac depressant factor in septic shock. J Cardiac Surg 2002;17:328–35.
[20] Ihara T, Ikeda U, Tate Y, Ishibashi S, Shimada K. Positive inotropic effects of adrenomedullin on rat papillary muscle. Eur J Pharmacol 2000;390:167–72.
[21] Ikeda U, Kanbe T, Kawahara Y, Yokoyama M, Shimada K. Adrenomedullin augments inducible nitric oxide synthase expression in cytokine-stimulated cardiac myocytes. Circulation 1996;94:2560–5.
[22] Ikenouchi H, Kangawa K, Matsuo H, Hirata Y. Negative inotropic effect of adrenomedullin in isolated adult rabbit cardiac ventricular myocytes. Circulation 1997;95:2318–24.
[23] Ishimitsu T, Nishikimi T, Saito Y, Kitamura K, Eto T, Kangawa K, et al. Plasma levels of adrenomedullin, a newly identified hypotensive peptide, in patients with hypertension and renal failure. J Clin Invest 1994;94:2158–61.
[24] Jougasaki M, Wei CM, McKinley LJ, Burnett Jr JC. Elevation of circulating and ventricular adrenomedullin in human congestive heart failure. Circulation 1995;92:286–9.
[25] Kapas S, Catt KJ, Clark AJ. Cloning and expression of cDNA encoding a rat adrenomedullin receptor. J Biol Chem 1995;270:25344–7.
[26] Kelly RA, Balligand JL, Smith TW. Nitric oxide and cardiac function. Circ Res 1996;79:363–80.
[27] Kitamura K, Kangawa K, Kawamoto M, Ichiki Y, Nakamura S, Matsuo H, et al. Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. Biochem Biophys Res Commun 1993;192:553–60.
[28] Kitamura K, Sakata J, Kangawa K, Kojima M, Matsuo H, Eto T. Cloning and characterization of cDNA encoding a precursor for human adrenomedullin. Biochem Biophys Res Commun 1993;194:720–5.
[29] Lainchbury JG, Meyer DM, Jougasaki M, Burnett Jr JC, Redfield MM. Effects of adrenomedullin on load and myocardial performance in normal and heart-failure dogs. Am J Physiol 2000;279:H1000–6.
[30] Leite-Moreira AF. Current perspectives in diastolic dysfunction and diastolic heart failure. Heart (Brit Cardiac Soc) 2006;92:712–8.
[31] Leite-Moreira AF, Bras-Silva C, Pedrosa CA, Rocha-Sousa AA. ET-1 increases distensibility of acutely loaded myocardium: a novel ETA and Na+/H+ exchanger-mediated effect. Am J Physiol 2003;284:H1332–9.
[32] Leite-Moreira AF, Castro-Chaves P, Pimentel-Nunes P, Lima-Carneiro A, Guerra MS, Soares JB, et al. Angiotensin II acutely decreases myocardial stiffness: a novel AT1, PKC and Na+/H+ exchanger-mediated effect. Brit J Pharmacol 2006;147:690–7.
[33] McLatchie LM, Fraser NJ, Main MJ, Wise A, Brown J, Thompson N, et al. RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. Nature 1998;393:333–9.
[34] Mittra S, Bourreau JP. Gs and Gi coupling of adrenomedullin in adult rat ventricular myocytes. Am J Physiol 2006;290:H1842–7.
[35] Mittra S, Hyvelin JM, Shan Q, Tang F, Bourreau JP. Role of cyclooxygenase in ventricular effects of adrenomedullin: is adrenomedullin a double-edged sword in sepsis? Am J Physiol 2004;286:H1034–42.
[36] Miyao Y, Nishikimi T, Goto Y, Miyazaki S, Daikoku S, Morii I, et al. Increased plasma adrenomedullin levels in patients with acute myocardial infarction in proportion to the clinical severity. Heart (Brit Cardiac Soc) 1998;79:39–44.
[37] Morgan JP. Abnormal intracellular modulation of calcium as a major cause of cardiac contractile dysfunction. N Engl J Med 1991;325:625–32.
[38] Mukherjee R, Multani MM, Sample JA, Dowdy KB, Zellner JL, Hoover DB, et al. Effects of adrenomedullin on human myocyte contractile function and beta-adrenergic response. J Cardiovasc Pharmacol Therap 2002;7:235–40.
[39] Nagaya N, Satoh T, Nishikimi T, Uematsu M, Furuichi S, Sakamaki F, et al. Hemodynamic, renal, and hormonal effects of adrenomedullin infusion in patients with congestive heart failure. Circulation 2000;101:498–503.
[40] Nicholls MG, Charles CJ, Lainchbury JG, Lewis LK, Rademaker MT, Richards AM, et al. Adrenomedullin in heart failure. Hypertens Res 2003;(26 Suppl.):S135–40.
[41] Nishikimi T, Horio T, Yoshihara F, Nagaya N, Matsuo H, Kangawa K. Effect of adrenomedullin on cAMP and cGMP levels in rat cardiac myocytes and nonmyocytes. Eur J Pharmacol 1998;353:337–44.
[42] Nishikimi T, Yoshihara F, Mori Y, Kangawa K, Matsuoka H. Cardioprotective effect of adrenomedullin in heart failure. Hypertens Res 2003;(26 Suppl.):S121–7.
[43] Nishimatsu H, Suzuki E, Nagata D, Moriyama N, Satonaka H, Walsh K, et al. Adrenomedullin induces endothelium-dependent vasorelaxation via the phosphatidylinositol 3-kinase/Akt-dependent pathway in rat aorta. Circ Res 2001;89:63–70.
[44] Oie E, Vinge LE, Yndestad A, Sandberg C, Grogaard HK, Attramadal H. Induction of a myocardial adrenomedullin signaling system during ischemic heart failure in rats. Circulation 2000;101:415–22.
[45] Oya H, Nagaya N, Furuichi S, Nishikimi T, Ueno K, Nakanishi N, et al. Comparison of intravenous adrenomedullin with atrial natriuretic peptide in patients with congestive heart failure. Am J Cardiol 2000;86:94–8.
[46] Pan CS, Jin SJ, Cao CQ, Zhao J, Zhang J, Wang X, et al. The myocardial response to adrenomedullin involves increased cAMP generation as well as augmented Akt phosphorylation. Peptides 2007;28:900–9.
[47] Parkes DG. Cardiovascular actions of adrenomedullin in conscious sheep. Am J Physiol 1995;268:H2574–8.
[48] Paulus WJ, Shah AM. NO and cardiac diastolic function. Cardiovasc Res 1999;43:595–606.
[49] Paulus WJ, Tschope C, Sanderson JE, Rusconi C, Flachskampf FA, Rademakers FE, et al. How to diagnose diastolic heart failure: a consensus statement on the diagnosis of heart failure with normal left ventricular ejection fraction by the Heart Failure and Echocardiography Associations of the European Society of Cardiology. Eur Heart J 2007.
[50] Paulus WJ, Vantrimpont PJ, Shah AM. Acute effects of nitric oxide on left ventricular relaxation and diastolic distensibility in humans. Assessment by bicoronary sodium nitroprusside infusion. Circulation 1994;89:2070–8.
[51] Paulus WJ, Vantrimpont PJ, Shah AM. Paracrine coronary endothelial control of left ventricular function in humans. Circulation 1995;92:2119–26.
[52] Perret M, Broussard H, LeGros T, Burns A, Chang JK, Summer W, et al. The effect of adrenomedullin on the isolated heart. Life Sci 1993;53:PL377–9.
[53] Rademaker MT, Charles CJ, Lewis LK, Yandle TG, Cooper GJ, Coy DH, et al. Beneficial hemodynamic and renal effects of adrenomedullin in an ovine model of heart failure. Circulation 1997;96:1983–90.

[54] Saetrum Opgaard O, de Vries R, Tom B, Edvinsson L, Saxena PR. Positive inotropy of calcitonin gene-related peptide and amylin on porcine isolated myocardium. Eur J Pharmacol 1999;385:147–54.
[55] Saetrum Opgaard O, Hasbak P, de Vries R, Saxena PR, Edvinsson L. Positive inotropy mediated via CGRP receptors in isolated human myocardial trabeculae. Eur J Pharmacol 2000;397:373–82.
[56] Sexton PM, Albiston A, Morfis M, Tilakaratne N. Receptor activity modifying proteins. Cell Signal 2001;13:73–83.
[57] Shah AM, MacCarthy PA. Paracrine and autocrine effects of nitric oxide on myocardial function. Pharmacol Therap 2000;86:49–86.
[58] Shimekake Y, Nagata K, Ohta S, Kambayashi Y, Teraoka H, Kitamura K, et al. Adrenomedullin stimulates two signal transduction pathways, cAMP accumulation and Ca^{2+} mobilization, in bovine aortic endothelial cells. J Biol Chem 1995;270:4412–7.
[59] Stangl V, Dschietzig T, Bramlage P, Boye P, Kinkel HT, Staudt A, et al. Adrenomedullin and myocardial contractility in the rat. Eur J Pharmacol 2000;408:83–9.
[60] Szokodi I, Kinnunen P, Tavi P, Weckstrom M, Toth M, Ruskoaho H. Evidence for cAMP-independent mechanisms mediating the effects of adrenomedullin, a new inotropic peptide. Circulation 1998;97:1062–70.
[61] Terata K, Miura H, Liu Y, Loberiza F, Gutterman DD. Human coronary arteriolar dilation to adrenomedullin: role of nitric oxide and K(+) channels. Am J Physiol 2000;279:H2620–6.
[62] Udawela M, Hay DL, Sexton PM. The receptor activity modifying protein family of G protein coupled receptor accessory proteins. Semin Cell Develop Biol 2004;15:299–308.
[63] Zimmermann U, Fischer JA, Muff R. Adrenomedullin and calcitonin gene-related peptide interact with the same receptor in cultured human neuroblastoma SK-N-MC cells. Peptides 1995;16:421–4.