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Positive inotropic effects of adrenomedullin on rat papillary muscle

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Abstract

Adrenomedullin is a peptide recently isolated from pheochromocytoma that has vasorelaxant and long-lasting hypotensive activities. Plasma levels of adrenomedullin are elevated in patients with congestive heart failure, but the effects of adrenomedullin on the cardiac function are unclear. We, thus, investigated the effects of adrenomedullin on the contraction of rat papillary muscles. We measured the isometric tension and cAMP contents of isolated rat papillary muscles. Adrenomedullin exhibited concentration-dependent inotropic effects. Adrenomedullin also significantly increased intracellular contents of cAMP. Addition of the calcitonin gene-related peptide (CGRP) receptor antagonist inhibited both contractile force and cAMP generation of papillary muscles stimulated by adrenomedullin. The adrenomedullin-induced inotropic effect was further increased in the presence of the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (IBMX), while the effect was significantly suppressed by KT5720 and Rp-8-bromoadenosine-3′,5′-cyclic monophosphorothioate (Rp-8-Br-cAMPS), protein kinase A inhibitors. These results indicate that adrenomedullin has positive inotropic effects on the heart, at least partially through a cAMP-dependent pathway.

Keywords: Adrenomedullin; Papillary muscle; Contractility; cAMP

1. Introduction

Adrenomedullin is a newly discovered, potent hypotensive peptide that was originally isolated from human pheochromocytoma (Kitamura et al., 1993). This peptide consists of 52 amino acids and one intramolecular disulfide bond (Richards et al., 1996; Schell et al., 1996) and is produced in several tissues including the adrenal medulla, heart, lung, and kidney (Ichiki et al., 1994; Kitamura et al., 1994). When injected intravenously into rats, adrenomedullin elicits a strong, long-lasting hypotension, a consequence of vasodilation in the resistance artery (Ishiyama et al., 1993; Nuki et al.,1993). Adrenomedullin may also act as paracrine and autocrine factors in the regulation of cardiac function, because high adrenomedullin mRNA expression, a considerable amount of adrenoedullin-like immunoreactivity, and a high level of ^{125}I-adrenomedullin binding have been found in the heart (Ishiyama et al., 1993; Sakata et al., 1993). We also observed that adrenomedullin increases intracellular cAMP levels of cardiac myocytes (Ikeda et al., 1996). Thus, the heart could be a target organ of adrenomedullin.

Elevated plasma levels of adrenomedullin have been noted in patients with congestive heart failure (Jougasaki et al., 1995; Nishikimi et al., 1995) and acute myocardial infarction (Kobayashi et al., 1996). Jougasaki et al. (1995) reported that immunohistochemical staining for adrenomedullin is significantly increased in the failing human ventricle. We have previously reported that plasma adrenomedullin levels are increased in patients with mitral stenosis, and percutaneous mitral valvuloplasty significantly decreases this elevation (Yamamoto et al., 1998). These observations suggest that circulating or locally produced adrenomedullin within the heart may modulate myocardial contractility, but there has been no agreement about the effects of adrenomedullin on myocardial function. Szokodi et al. (1996) reported that adrenomedullin exerts a direct inotropic effect on isolated perfused rat hearts, while Ikenouchi et al. (1997) reported that adrenomedullin has a negative inotropic effect on isolated adult rabbit cardiac ventricular myocytes. To clarify the effects of adrenomedullin on cardiac function, we investi-

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gated its effects on isometric contraction and intracellular cAMP levels of isolated rat papillary muscles.

2. Materials and methods

2.1. Reagents

Recombinant rat adrenomedullin, calcitonin gene-related peptide (CGRP) and the CGRP receptor antagonist CGRP-(8-37) were purchased from Peptide Institute (Osaka, Japan). 3-Isobutyl-1-methyl-xanthine (IBMX) was obtained from Sigma (St.Louis, MO). KT5720 was obtained from Kyowa Medex (Tokyo, Japan). Rp-8-bromoadenosine-3′,5′-cyclic monophosphorothioate (Rp-8-Br-cAMPS) was purchased from Biolog Life Science Institute (Bremen, Germany). All other chemicals used were of the highest grade commercially available.

2.2. Recording of mechanical responses

Male Sprague–Dawley rats (12–13 weeks old) were anaesthetized by intraperitoneal administration of pentobarbital (100 mg/kg). Hearts were quickly removed and retrogradely washed through the aorta with oxygenated Krebs–Henseleit solution, pH 7.4. The composition of the buffer was (in mM): NaCl 113.8; KCl 4.7; $CaCl_2$ 2.5; KH_2PO_4 1.2; $MgSO_4$ 1.1; $NaHCO_3$ 22; glucose 11 at 27°C. Thin papillary muscles were dissected and mounted horizontally in a muscle chamber. One end of the preparation was attached to a fixed hook and the other was connected to the arm of a tension transducer (TB611T, Nihon Kohden Kogyo, Tokyo, Japan) with cotton thread, and tension was displayed on a recorder with a built-in preamplifier (AP600G; Nihon Kohden Kogyo). A pair of platinum black electrodes was placed in parallel with the preparation for electrical stimulation. The preparation was continuously stimulated by rectangular pulses at 0.5 Hz; the strength of the stimulation was 1.5 times threshold. Before the start of the experiment, the preparation was stretched to L_{max}, the length at which developed tension becomes maximal.

2.3. Measurement of cAMP levels

For determination of intracellular cAMP levels, 0.5 mM IBMX, a cyclic nucleotide phosphodiesterase inhibitor, was added 30 min before the adrenomedullin to prevent breakdown of accumulated cAMP. After incubation with adrenomedullin for 15 min, tissue sections were rapidly frozen in liquid nitrogen. Frozen tissues were then homogenized in cold 6% (w/v) trichloroacetic acid, and centrifuged at $2500 \times g$ for 15 min at room temperature. The supernatants were decanted, and after 0.05 ml of 50 mM sodium acetate was added to each tube. The pellets were dissolved in 0.2 ml of 1% sodium dodecyl sulfate (SDS) and kept at 4°C until protein assay. Intracellular cAMP contents were measured with a commercial enzyme immunoassay kit with the manufacturer's high-sensitivity acetylation protocol (Amersham, Buckinghamshire, England). The lower limit of detection was 2 fmol/well. The values were normalized to the protein content of each preparation.

2.4. Statistical analysis

Results of experiments are expressed as mean ± S.E.M. Comparisons were made by one-way analysis of variance combined with Scheffe's test. P values < 0.05 were regarded as significant.

3. Results

3.1. Effect of adrenomedullin on papillary muscle contraction

Adrenomedullin was applied to the preparation cumulatively after the equilibration of the preparations in Krebs–Henseleit solution for over 1 h. A representative tracing of

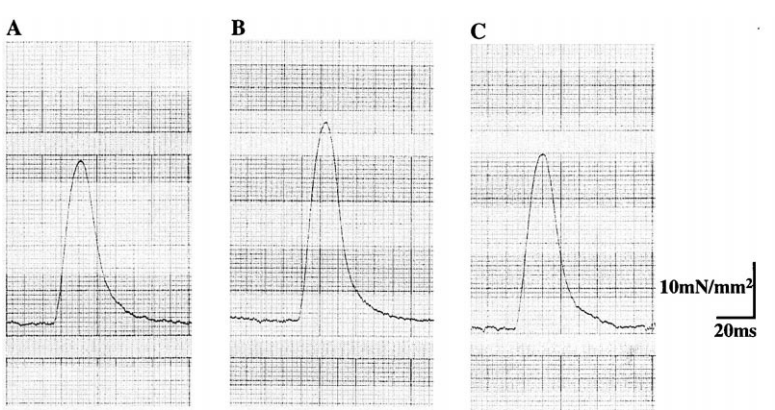

Fig. 1. Recordings show the effects of adrenomedullin on the force of rat papillary muscles electrically driven at 0.5 Hz at 27°C. (A) control, (B) 20 min after application of adrenomedullin (10^{-7} M), (C) 20 min after application of adrenomedullin (10^{-7} M) plus CGRP-(8-37) (10^{-6} M).

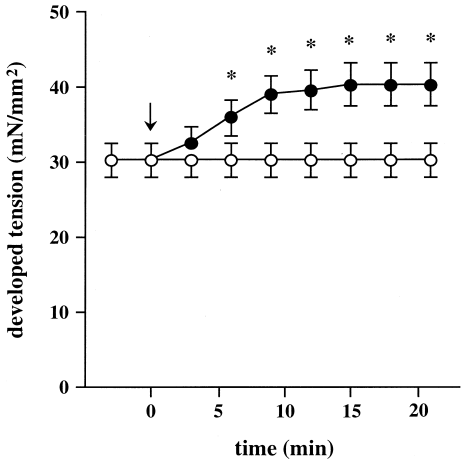

Fig. 2. Time-course of the effects of adrenomedullin on the force of rat papillary muscles. The force of rat papillary muscles electrically driven at 0.5 Hz at 27°C was measured in the presence (closed circles) or absence (open circles) of 10^{-7} M adrenomedullin. An arrow indicates the time of adrenomedullin administration. Data represent means ± S.E.M. of five experiments. * Significantly different ($p < 0.05$) from the control without adrenomedullin.

the effect of 10^{-7} M adrenomedullin on the papillary muscle contractility is shown in Fig. 1. The elevation of the developed tension by adrenomedullin was gradual, and maximum increases were seen 15–20 min after addition of adrenomedullin (Fig. 2). Adrenomedullin did not significantly influence the resting tension of papillary muscles.

As shown in Fig. 3, adrenomedullin produced a dose-dependent increase in developed tension. The peak tension increased significantly up to 134% of the control with 10^{-7} M adrenomedullin. This increase was significantly inhibited by CGRP-(8-37), a CGRP receptor antagonist.

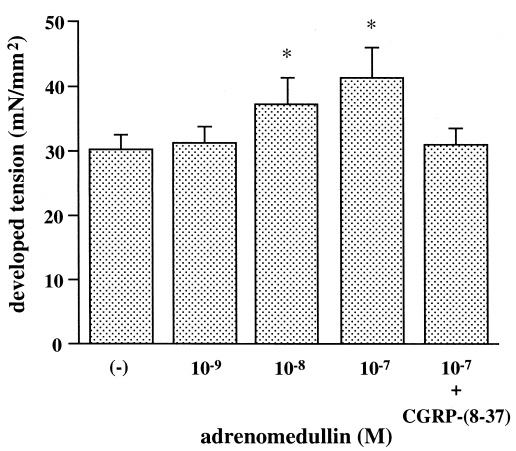

Fig. 3. Dose-dependent effects of adrenomedullin on the force of rat papillary muscles. The final concentration of adrenomedullin was increased cumulatively from 10^{-9} to 10^{-7} M. CGRP-(8-37) (10^{-6} M) was also added to papillary muscles stimulated with 10^{-7} M adrenomedullin. Developed tension was measured when the effects reached the equilibrium state (15–20 min after drug application). Data represent means ± S.E.M. of five experiments. * Significantly different from the control without adrenomedullin.

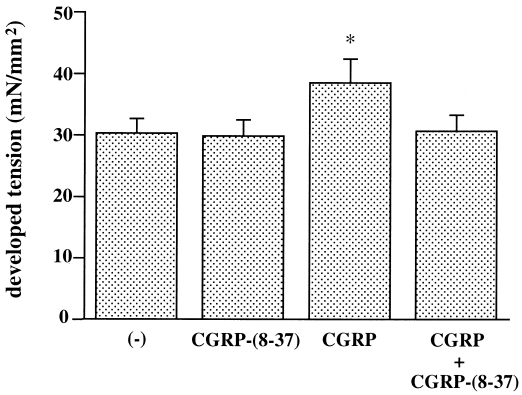

Fig. 4. Effects of CGRP on the force of rat papillary muscles. CGRP (10^{-7} M) with or without CGRP-(8-37) (10^{-6} M) was also added to papillary muscles. Developed tension was measured when the effects reached the equilibrium state (15–20 min after drug application). Data represent means ± S.E.M. of four experiments. * Significantly different from the control without CGRP.

These results suggest that the effect of adrenomedullin is mediated by CGRP receptors. We, thus, investigated the effect of CGRP on developed tension. As shown in Fig. 4, 10^{-7} M CGRP significantly increased developed tension, and this increase was inhibited by CGRP-(8-37). CGRP-(8-37) alone showed no effect on developed tension.

3.2. Involvement of cAMP in the action of adrenomedullin

We then investigated the mechanism of the inotropic effect of adrenomedullin on the contraction of rat papillary muscles. Recently, we have shown that adrenomedullin stimulates cAMP formation in cultured rat cardiac my-

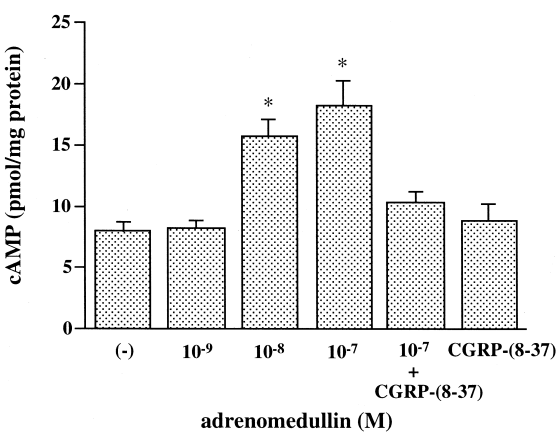

Fig. 5. Dose-dependent effects of adrenomedullin on intracellular cAMP levels in rat papillary muscles. Rat papillary muscles were incubated for 15 min with final 10^{-9} to 10^{-7} M adrenomedullin in the presence of 0.5 mM IBMX added 30 min before the addition of adrenomedullin. CGRP-(8-37) (10^{-6} M) was also added to papillary muscles. Intracellular cAMP levels were measured as described in Section 2, and the values were normalized to the protein content per preparation. Data represent mean ± S.E.M. ($n = 4$). * Significantly different from the control without adrenomedullin.

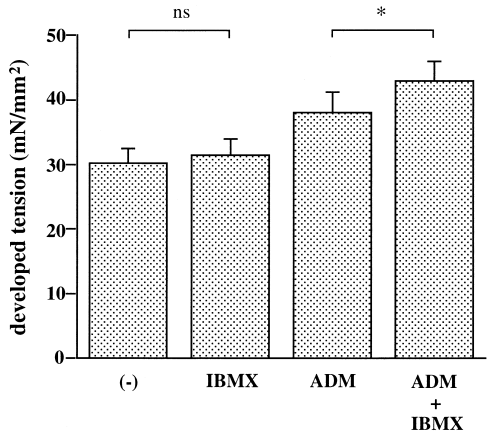

Fig. 6. Influence of IBMX on the inotropic effect of adrenomedullin. Rat papillary muscles were driven at 0.5 Hz at 27°C with 0.5 mM IBMX in the prescence or absence of 10^{-8} M adrenomedullin (ADM). Data represent mean ± S.E.M. ($n = 4$). $^{*}P < 0.05$.

ocytes (Ikeda et al., 1996); therefore, we speculate the involvement of a cAMP-dependent pathway in the effect of adrenomedullin. Incubation with adrenomedullin for 15 min increased cAMP levels of papillary muscles, and this adrenomedullin-induced cAMP elevation was significantly inhibited in the presence of CGRP-(8-37) (Fig. 5).

We then studied effects of the phosphodiesterase inhibitor IBMX and protein kinase A inhibitor KT5720 and Rp-8-Br-cAMPS (Gjertsen et al., 1995) on contraction of papillary muscles stimulated by adrenomedullin. As shown in Fig. 6, IBMX alone showed no effect on the contraction, while the adrenomedullin-induced contraction was further increased in the presence of IBMX. On the other hand, KT5720 and Rp-8-Br-cAMPS significantly suppressed adrenomedullin-induced contraction (Fig. 7). These data further suggest that adrenomedullin increases the contractile force of rat papillary muscles through a cAMP-dependent process.

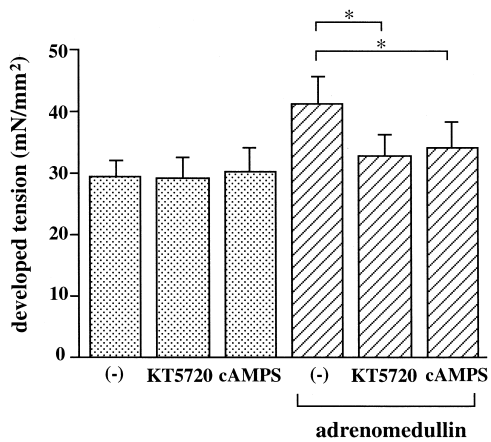

Fig. 7. Influence of KT5720 and Rp-8-Br-cAMPS on the inotropic effect of adrenomedullin. Rat papillary muscles were driven at 0.5 Hz at 27°C with KT5720 (10^{-7} M) or Rp-8-Br-cAMPS (10^{-6} M) in the prescence (hatched bars) or absence (dotted bars) of 10^{-7} M adrenomedullin (ADM). Data represent mean ± S.E.M. ($n = 4$). $^{*}P < 0.05$.

4. Discussion

Previously, Ikenouchi et al. (1997) reported that adrenomedullin had a negative inotropic effect on isolated adult rabbit cardiac ventricular myocytes mediated through the L-arginine-nitric oxide (NO) pathway. On the other hand, adrenomedullin has been reported to increase cardiac output and left ventricular contractility of conscious sheep (Parkes, 1995; Parkes and May, 1997) and exert a direct inotropic effect on isolated perfused rat hearts (Szokodi et al., 1996). In this study, we showed that adrenomedullin has a concentration-dependent inotropic effect on rat papillary muscles. Addition of N^{G}-monomethyl-L-arginine, a NO synthase inhibitor, had no effect on adrenomedullin-induced contraction (data not shown), suggesting that a NO pathway is not involved. It is difficult to explain the difference between our results and those of Ikenouchi et al. (1997), but differences in the experimental preparation or species used may be involved.

Adrenomedullin increases intracellular cAMP levels in various types of cells including kidney cells (Chini et al., 1995), endothelial cells (Shimekake et al., 1995), vascular smooth muscle cells (Eguchi et al., 1994; Ishizaka et al., 1994), and platelets. Similarly, adrenomedullin has been reported to stimulate cAMP formation in isolated cardiac myocytes (Ikeda et al., 1996; Sato et al., 1997). These observations suggest that activation of the adenylate cyclase–cAMP system, which is one of the major pathways for the stimulation of cardiac contractility in the mammalian heart (Morgan, 1991), may mediate the cardiac effects of adrenomedullin. Indeed, intracellular cAMP contents of the rat papillary muscle were elevated by adrenomedullin in a concentration-dependent manner, suggesting cAMP production and subsequent protein kinase A activation are involved in the inotropic effect of adrenomedullin. We, thus, studied the effects of the protein kinase A inhibitor KT5720 on adrenomedullin-induced contraction. KT5720 is extremely selective for protein kinase A (K_i value for protein kinase A is 60 nM, and those values for protein kinase G, protein kinase C, and myosin light chain kinase are > 2000 nM) (Kase et al., 1987). Addition of only KT5720 at a final concentration of 10^{-7} M had no influence on the developed tension of rat papillary muscles, but it did inhibit subsequent adrenomedullin-induced positive inotropic effects. We also found that Rp-8-Br-cAMPS, a competitive inhibitor of protein kinase A, inhibited the effect of adrenomedullin.

Previously, it has been shown that various responses to adrenomedullin can be blocked by the CGRP receptor antagonist CGRP-(8-37), suggesting that adrenomedullin interacts with CGRP receptors (Entzeroth et al., 1995; Nakamura et al., 1995). Owji et al. (1995) had reported

that there are at least two subtypes of adrenomedullin receptors, CGRP receptors and specific adrenomedullin receptors. They speculated that the increases in cAMP levels induced by adrenomedullin may be due to cross-activation of CGRP receptors, whereas signal transduction via specific adrenomedullin receptors may not involve the cAMP pathway. Previously, Bell and McDermott (1995) and Huang et al. (1999) reported that CGRP enhanced rat ventricular myocyte contraction, while Ikenouchi et al. (1997) reported negative inotropic effects of CGRP on adult rabbit ventricular myocytes. In the present study, CGRP exhibited inotropic effects, and both effects of adrenomedullin on the developed tension and cAMP contents were significantly inhibited by CGRP-(8-37), suggesting that the effects of adrenomedullin on the rat papillary muscle contraction may be mediated via CGRP receptors.

Saturation analysis revealed a dissociation constant for adrenomedullin of $0.41 \pm 0.14 \times 10^{-9}$ M (mean ± S.D.) in the rat heart (Owji et al., 1995). According to Ichiki et al. (1994) and Sakata et al. (1994), mean plasma concentrations of adrenomedullin in rats and humans are $3.6 \pm 0.3 \times 10^{-9}$ and $3.3 \pm 0.4 \times 10^{-9}$ M, respectively. In patients with acute myocardial infarction or congestive heart failure, plasma levels of adrenomedullin become increased by several folds (Jougasaki et al., 1995; Nishikimi et al., 1995; Kobayashi et al., 1996). Thus, clinical concentrations of adrenomedullin were sufficient to achieve its effects on cardiac contractility. In addition, local adrenomedullin levels in heart tissues may be much higher than plasma adrenomedullin concentrations because adrenomedullin has been shown to be synthesized in the heart (Ichiki et al., 1994).

Recently, Szokodi et al. (1998) reported that adrenomedullin enhanced contraction of the isolated perfused rat heart preparation via cAMP-independent mechanism. In their study, adrenomedullin did not increased ventricular cAMP contents of the perfused hearts, although we (Ikeda et al., 1996) and Sato et al. (1997) have observed that adrenomedullin stimulates cAMP formation in cardiac myocytes. The differences in the experimental preparation may cause the discrepancy in the results. Another possibility for difference between the results of this study and those of Szokodi et al. (1998) may be the dose of adrenomedullin (10–100 vs. 0.1–1 nM). Adrenomedullin at lower doses may enhance contractility via cAMP-independent mechanisms, and at higher doses, stimulate cAMP formation and induce the positive inotropic effect.

In conclusion, these results show that adrenomedullin has an inotropic effect on the rat heart, at least partially through a cAMP-dependent pathway. Taking into account the potent inotropic effect of adrenomedullin and the increased ventricular production of adrenomedullin in the severely failing human myocardium, the present results are consistent with the hypothesis that adrenomedullin may play a role in compensatory mechanisms against deterioration of cardiac performance in congestive heart failure.

Acknowledgements

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References

Bell, D., McDermott, B.J., 1995. Activity of amylin at $CGRP_1$-preferring receptors coupled to positive contractile response in rat ventricular cardiomyocytes. Regul. Pept. 60, 125–133.

Chini, E.N., Choi, E., Grande, J.P., Burnett, J.C., Dousa, T.P., 1995. Adrenomedullin suppresses mitogenesis in rat mesangial cells via cAMP pathway. Biochem. Biophys. Res. Commun. 215, 868–873.

Eguchi, S., Hirata, Y., Kano, H., Sato, K., Watanabe, Y., Watanabe, T.X., Nakjima, K., Sakakibara, S., Marumo, F., 1994. Specific receptors for adrenomedullin in cultured rat vascular smooth muscle cells. FEBS Lett. 340, 226–230.

Entzeroth, M., Doods, H.N., Wieland, H.A., Wienen, W., 1995. Adrenomedullin mediates vasodilation via CGRP1 receptors. Life Sci. 56, 19–25.

Gjertsen, B.T., Mellgren, G.S., Otten, A., Maronde, E., Genieser, H.G., Jastorff, B., Vintermyr, O.K., McKnight, G.S., Doskeland, S.O., 1995. Novel (Rp)-cAMPS analogs as tools for inhibition of cAMP-kinase in cell culture: basal cAMP-kinase activity modulates interleukin-1β action. J. Biol. Chem. 270, 20599–20607.

Huang, M.H., Knight, P.R. III, Izzo, J.L. Jr., 1999. Ca^{2+}-induced Ca^{2+} release involved in positive inotropic effect mediated by CGRP in ventricular myocytes. Am. J. Physiol. 276, R259–R264.

Ichiki, Y., Kitamura, K., Kangawa, K., Kawamoto, M., Matsuo, H., Eto, H., 1994. Distribution and characterization of immunoreactive adrenomedullin in human tissue and plasma. FEBS Lett. 338, 6–10.

Ikeda, U., Kanbe, T., Kawahara, Y., Yokoyama, M., Shimada, K., 1996. Adrenomedulin augments inducible nitric oxide synthase in cytokine-stimulated cardiac myocytes. Circulation 94, 2560–2565.

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.

Ishizaka, Y., Tanaka, M., Kitamura, K., Kangawa, K., Minamino, N., Matsuo, H., Eto, T., 1994. Adrenomedullin stimulates cyclic AMP formation in rat vascular smooth muscle cells. Biochem. Biophys. Res. Commun. 200, 642–646.

Jougasaki, M., Wei, C., McKinley, L., Burnett, J., 1995. Elevation of circulating and ventricular adrenomedullin in human congestive heart failure. Circulation 92, 286–289.

Kase, H., Iahashi, K., Nakanishi, S., Matsuda, Y., Yamada, K., Takahashi, M., Murakata, C., Sato, A., Kaneko, M., 1987. K-252 compounds, novel and potent inhibitors of protein kinase C and cyclic nucleotide-dependent protein kinase. Biochem. Biophys. Res. Commun. 142, 436–440.

Kitamura, K., Ichiki, Y., Tanaka, M., Kawamoto, M., Emura, J., Sakakibara, S., Kangawa, K., Matsuo, H., Eto, T., 1994. Immunoreactive adrenomedullin in human plasma. FEBS Lett. 341, 288–290.

Kitamura, K., Kangawa, K., Kawamoto, M., Ichiki, Y., Nakamura, S., Matsuo, H., Eto, T., 1993. Adrenomedullin: a novel hypotensive

peptide isolated from human pheochromocytoma. Biochem. Biophys. Res. Commun. 192, 553–560.

Kobayashi, K., Kitamura, K., Hirayama, N., Date, H., Kashigawa, T., Ikushima, I., Handa, Y., Nagatomo, Y., Takenaga, M., Ishikawa, T., Imamura, T., Koiwaya, Y., Eto, T., 1996. Increased plasma adrenomedullin in acute myocardial infarction. Am. Heart J. 131, 676–680.

Morgan, J.P., 1991. Abnormal intracellular modulation of calcium as a major cause of cardiac contractile dysfunction. N. Engl. J. Med. 325, 625–632.

Nakamura, K., Toda, H., Terasako, K., Kakuyama, M., Hatano, Y., Mora, K., Kangawa, K., 1995. Vasodilator effect of adrenomedullin in isolated arteries of the dog. Jpn. J. Pharmacol. 67, 259–262.

Nishikimi, T., Saito, Y., Kitamura, K., Ishimitsu, T., Eto, T., Kangawa, K., Matsuo, H., Omae, T., Matsuoka, H., 1995. Increased plasma levels of adrenomedullin in patients with heart failure. J. Am. Coll. Cardiol. 26, 1424–1431.

Nuki, C., Kawasaki, H., Kitamura, K., Takegawa, M., Kangawa, K., Eto, T., Wada, A., 1993. Vasodilator effect of adrenomedullin and calcitonin gene-related peptide receptors in rat mesenteric vascular beds. Biochem. Biophys. Res. Commun. 196, 245–251.

Owji, A.A., Smith, D.M., Coppock, H.A., Morgan, D.G.A., Bhogal, R., Ghatel, M.A., Bloom, S.R., 1995. An abundant and specific binding site for the novel vasodilator adrenomedullin in the rat. Endocrinology 136, 2127–2134.

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

Parkes, D.G., May, C.N., 1997. Direct cardiac and vascular actions of adrenomedullin in conscious sheep. Br. J. Pharmacol. 120, 1179–1185.

Richards, A.M., Nichols, M.G., Lewis, L., 1996. Lainchbury JG. Adrenomedullin Clin. Sci. 91, 3–16.

Sakata, J., Shimokubo, T., Kitamura, K., Nakamura, S., Kangawa, K., Matsuo, H., Eto, T., 1993. Molecular cloning and biological activities of rat adrenomedullin, a hypotensive peptide. Biochem. Biophys. Res. Commun. 195, 921–927.

Sakata, J., Shimokubo, T., Kitamura, K., Nishizono, M., Iehiki, Y., Kangawa, K., Matsuno, H., Eto, T., 1994. Distribution and characterization of immunoreactive rat adrenomedullin in tissue and plasma. FEBS Lett. 352, 105–108.

Sato, A., Canny, B.J., Autelitno, D.L., 1997. Adrenomedullin stimulates cAMP accumulation and inhibits atrial natriuretic peptide gene expression in cardiomyocytes. Biochem. Biophys. Res. Commun. 230, 311–314.

Schell, D.A., Vari, R.C., Samson, W.K., 1996. Adrenomedullin: a newly discovered hormone controlling fluid and electrolyte homeostasis. Trends Endocrinol. 7, 7–13.

Shimekake, Y., Nagata, L., Ohta, S., Kambayashi, Y., Teraoka, H., Kitamura, K., Eto, T., Kangawa, K., Matsuo, H., 1995. Adrenomedullin stimulates two signal transduction pathways, cAMP accumulation and Ca^{2+} mobilization, in bovine aortic endothelial cells. J. Biol. Chem. 270, 4412–4417.

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., Weckstrom, M., Toth, M., Ruskoaho, H., 1998. Evidence for cAMP-independent mechanisms mediating the effects of adrenomedullin, a new inotropic peptide. Circulation 97, 1062–1070.

Yamamoto, K., Ikeda, U., Sekiguchi, H., Shimada, K., 1998. Plasma levels of adrenomedullin in patients with mitral stenosis. Am. Heart J. 135, 542–549.