Endothelium-Independent Relaxation by Adrenomedullin in Pregnant Rat Mesenteric Artery: Role of cAMP-Dependent Protein Kinase A and Calcium-Activated Potassium Channels

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ABSTRACT
The mechanisms of relaxation of adrenomedullin were investigated in isolated mesenteric artery from pregnant rats. Adrenomedullin (1 nM–0.3 μM) produced concentration-dependent relaxation of endothelium-denuded mesenteric artery rings precontracted with norepinephrine at a concentration required to produce 70% of maximal response (ED70). The concentration-response curve of adrenomedullin was shifted to the right by adrenomedullin receptor antagonist adrenomedullin22–52 (10 μM) or calcitonin gene-related peptide8–37 (1 μM). Inhibition of adenylyl cyclase by 9-(tetrahydro-2-furanyl)-9H-purin-6-amine (SQ22536) (10 μM) or protein kinase A [Rp-cyclic adenosine monophosphorothioate (Rp-cAMP); 10 μM] reduced the adrenomedullin-induced relaxation to the same magnitude. Adrenomedullin increased the intracellular cAMP level from 0.38 ± 0.07 to 2.00 ± 0.47 pmol/mg tissues, which was completely inhibited by adrenomedullin22–52 (100 μM). Extracellular high potassium (80 mM), which inactivates the potassium channels, reduced the adrenomedullin-induced relaxation. Blockade of ATP-sensitive, voltage-gated, or inward rectifier potassium channels did not affect the adrenomedullin-induced relaxation. Blockade of calcium-activated K+ channels (KCa) by tetraethylammonium (1 mM) or iberiotoxin (100 nM) inhibited the adrenomedullin-induced relaxation, whereas there was no additional inhibition by SQ22536 or Rp-cAMP when KCa channels were already inhibited. In conclusion, this study provides evidence that cAMP-dependent protein kinase A and KCa channels seem to mediate as the cellular pathways in the adrenomedullin-induced endothelium-independent relaxation of mesenteric artery from pregnant rats.

Adrenomedullin is a novel vasodilatory peptide, which was originally discovered in human pheochromocytoma tissue in 1993 by Kitamura et al. (1993). Subsequent studies found adrenomedullin in several tissues, such as the adrenal medulla, the kidney, and the lungs (Ichiki et al., 1994), and revealed various biological actions, including vasodilatory activity. It was first reported that systemic intravenous injection of adrenomedullin in anesthetized rats elicited a potent hypotensive effect (Kitamura et al., 1993). Later, it was showed that the reduction in blood pressure by adrenomedullin was closely associated with the decrease in total peripheral resistance (Ishiyama et al., 1993). The increased plasma adrenomedullin levels during pregnancy (Jerat and Kaufman, 1998) suggest that adrenomedullin may play an important role in cardiovascular adaptation that occurs during pregnancy.

Many studies have investigated the mechanism of the vasodilatory effect of adrenomedullin; however, the results differed depending on the animal species and vascular preparation. For example, in perfused rat mesenteric vascular beds, administration of adrenomedullin induced endothelium-independent vasodilation (Nuki et al., 1993). On the other hand, adrenomedullin-induced rat mesenteric artery relaxation is reported to be endothelium-dependent (Champion et al., 2001). Adrenomedullin binds to specific receptors in endothelial cells and elicits endothelium-dependent vasorelaxation mediated by nitric oxide. According to Shimokaze et al. (1995), specific binding of adrenomedullin to bovine aortic endothelial cells was observed, and adrenomedullin induced intracellular cAMP accumulation in a dose-dependent manner. Adrenomedullin also dose-dependently in-
duced an increase in intracellular free calcium in endothelial cells by phospholipase C activation and inositol 1,4,5-trisphosphate formation, thereby activating nitric-oxide synthase (Shimekake et al., 1995). Nishimatsu et al. (2001) showed phosphatidylinositol 3-kinase/Akt-pathway involvement as another mechanism of endothelial nitric-oxide synthase activation. Some studies have shown that adrenomedullin caused endothelium-independent relaxation through the elevation of cAMP levels in vascular smooth muscle cells (Eguchi et al., 1994); whereas the downstream mechanism may vary depending on the vascular bed and species. The reported involvement of K+ channels in adrenomedullin-induced vasodilation is also puzzling, because adenosine triphosphatase-sensitive potassium (KATP) channels seem to mediate the adrenomedullin actions in dog coronary artery (Sabates et al., 1997) but not in rat mesenteric artery (Champion et al., 2001). Although most of the reports available dealt with endothelium-dependent mechanisms, not much is known regarding the endothelium-independent mechanisms in adrenomedullin-induced vascular relaxation. Thus, in this study, we proposed to elucidate the cAMP-dependent downstream mechanisms involved with respect to potassium channels in adrenomedullin-induced vasodilation in rat mesenteric artery using endothelium-denuded vessel. Because plasma adrenomedullin levels are elevated in normal pregnancy (Jerat and Kaufman, 1998) and adrenomedullin-induced hypotension is due to reduced total peripheral resistance, we used a resistance vessel (mesenteric artery) from pregnant rats for this study.

**Materials and Methods**

**Animals.** All procedures were approved by the Animal Care and Use Committee at the University of Texas Medical Branch in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Studies were performed in timed-pregnant Sprague-Dawley rats (250–280 g) obtained from Harlan (Houston, TX). All rats were maintained in the colony room with fixed photoperiod of 12-h light/12-h darkness, having access to water and rodent chow ad libitum. Rats were used on day 18 to day 20 of pregnancy.

**Myograph Mounting of Arteries.** The animals were killed by exsanguination under deep anesthesia with i.p. injection of ketamine (50 mg/kg) and xylazine (8 mg/kg). The mesentery artery was removed and placed in ice-cold physiological salt solution (PSS). The PSS contained the following composition: 114 mM NaCl, 4.7 mM KCl, 1.15 mM KH2PO4, 1.10 mM Na2HPO4, 1.18 mM MgSO4, 7 mM H2O, 15 mM NaHCO3, 1.15 mM CaCl2, and 5.0 mM glucose. Segments (~2 mm in length) of secondary branches of the superior mesenteric artery were isolated, cleaned of fat and connective tissue, mounted on a wire myograph (Kent Scientific, Litchfield, CT) using tungsten wires, and incubated for 15 to 30 min in PSS at 37°C, which was gassed with 95% air and 5% CO2 to maintain pH 7.4. The segment was then stretched to a length that was equivalent to a diameter of 225 to 250 μm and incubated for another 15 min. The tissue was activated to contract by the addition of 5 μM norepinephrine until reproducible responses were obtained. The relaxation responses were measured at cumulative doses of adrenomedullin between 10−8 and 3 × 10−7 M on vessel segments precontracted with effective concentration of norepinephrine required to produce 70% of maximal response (ED70) that was determined for each vessel.

Experiments were performed on endothelium-denuded arterial segments. The endothelium was removed by gentle rubbing of the intimal surface of the vessel with tungsten wire (size 1 μm) and confirmed by the failure of acetylcholine to relax the vascular segment precontracted by ED70 of norepinephrine. The viability of endothelium-denuded arterial rings was assessed by relaxation induced by sodium nitroprusside (1 μM). Time controls were run to establish the repeatability of adrenomedullin and the stability of the contractile response to norepinephrine.

**Experimental Protocol.** Concentration-response curves to adrenomedullin (10−8 to 3 × 10−5 M) were established on endothelium-denuded artery segments precontracted by ED70 of norepinephrine. The vessels were then washed thoroughly using PSS. After equilibration for 15 min, the arterial strips were incubated with various enzyme inhibitors, receptor antagonists, or channel blockers for 30 min before concentration-response curves to the adrenomedullin were constructed again. There are two types of receptors that mediate adrenomedullin actions. The association of calclotonin receptor-like receptor (CL) with receptor-activating-modifying protein (RAMP) subtype 2 (RAMP2) produces adrenomedullin receptor subtype 1 (AM1) selective to adrenomedullin that can be antagonized by the weak adrenomedullin peptide antagonist adrenomedullin22–52 and CL with RAMP, forms another adrenomedullin receptor subtype 2 (AM2) that can respond to both calclotonin gene-related peptide (CGRP) and adrenomedullin, which can be more potently antagonized by CGFR8–37 than by adrenomedullin22–52. To assess the receptor mediation, adrenomedullin receptor antagonists adrenomedullin22–52 (10 μM) and CGFR8–37 (1 μM) were used. To rule out the endothelial nitric-oxide synthase involvement in adrenomedullin-induced vasodilation, Nω-nitro-L-arginine methyl ester (l-LNAME) (100 μM) was used. To assess the role of adenylate cyclase and protein kinase A, we used 9-(tetrahydro-2-furan)-ML-purin-6-amine (SQ22536) (10 μM) and 8-bromoadenosine-3′,5′-cyclic monophosphorothioate, Rp-isomer (Rp-cAMP) (10 μM), respectively. To determine the involvement of potassium channels, respective blockers were used, viz. 10 μM glibenclamide (KATP), 10 μM barium chloride (KCl), 1 mM 4-aminopyridine (KV), 1 mM tetraethylammonium chloride (KIR), and 1 μM paxilline (maxi-K). To confirm the involvement of potassium channels, adrenomedullin-induced relaxation was done in tissues precontracted by 80 mM potassium PSS, a condition in which all of the potassium channels are inactivated.

**Measurement of cAMP Levels.** The basal and adrenomedullin-stimulated intracellular cAMP levels in mesenteric arterial strips were measured by radioimmunoassay using cAMP [3H] assay system (Amersham Biosciences, Inc., Little Chalfont, Buckinghamshire, UK). In brief, the mesenteric arterial arcade was carefully excised, removed, weighed and equilibrated for 1 h in 5 ml of Krebs’ buffer containing 100 μM isobutyl-1-methyl-xantine, a phosophodiesterase inhibitor (Sigma-Aldrich, St. Louis, MO), at 37°C aerated with 95% O2 and 5% CO2. After equilibration, tissues were incubated with 100 nM adrenomedullin for 2 min and rapidly frozen in liquid nitrogen and homogenized in 1.2 ml of 10% trichloroacetic acid. Tissues used to determine the antagonism by adrenomedullin antagonist, adrenomedullin22–52, were preincubated with 100 μM adrenomedullin22–52 for 30 min before adding adrenomedullin. In the cAMP measurements, since we used only a single (higher) concentration of adrenomedullin (0.3 μM), we chose 100 μM adrenomedullin22–52 because antagonists are generally used, at least approximately 2-fold more than the concentration of the agonists, to avoid the effect of the antagonist being surmounted by the higher concentration of agonist. The cAMP standard (2–128 fmol/tube) and samples (diluted 1:50) were acetylated by adding triethyamine/acetic anhydride [2:1 (v/v) 5 μl/tube]. Labeled cAMP bound to their respective antibodies were recovered by using magnetic beads with goat anti-rabbit IgG, and radioactivity was quantified in a gamma counter. cAMP levels are presented as picomole/milligram of tissue weight.

**Drugs Used.** Stock solutions of adrenomedullin (100 μM), norepinephrine (10 μM), adrenomedullin22–52 (100 μM), CGFR8–37 (1 μM), L-LNAME (0.1 M), SQ22536 (10 μM), Rp-cAMP (10 μM), BaCl2 (10 mM), 4-AP (1 M), tetraethylammonium (1 M), and iberiotoxin (100 μM) were dissolved in triple-distilled water aliquoted and stored at −80°C. Paxilline (1 mM) was dissolved in dimethyl sulfox-
ide. With the exception of adrenomedullin, adrenomedullin_{22-52} and CGRP_{8-37}, which were purchased from American Peptide Co., Inc. (Sunnyvale, CA), all other chemicals were purchased from Sigma-Aldrich.

### Statistical Analysis

Data are presented as mean ± S.E. Relaxation to adrenomedullin is expressed as 100 minus the percentage of the initial precontraction to norepinephrine. The data were analyzed by SigmaPlot 9.0 and Prism (GraphPad Software Inc., San Diego, CA) employing appropriate statistical tools. Means of different groups were analyzed by Student’s unpaired t test or one-way ANOVA followed by Bonferroni’s post test. Data were analyzed by two-way repeated measures ANOVA with Bonferroni post hoc test. Student’s unpaired t test or two-way repeated measures ANOVA with Bonferroni post hoc test. Student’s paired t test or two-way repeated measures ANOVA with Bonferroni post hoc test was used when comparisons were made between control and drug treatments in the same preparation. p < 0.05 was considered statistically significant. Individual concentration-response curves of adrenomedullin were subjected to linear regression analysis to determine EC_{50}, and data are expressed as pD_{2} (negative logarithm of the molar concentration of the agonist required to produce half-maximal response).

### Results

#### Effect of Adrenomedullin_{22-52} and CGRP_{8-37} on Adrenomedullin-Induced Vasodilation

The involvement of adrenomedullin receptors in adrenomedullin-induced vasodilation was assessed using the receptor antagonists adrenomedullin_{22-52} and CGRP_{8-37}. Incubation of arterial rings in adrenomedullin_{22-52} (10 μM) shifted the concentration-response curve of adrenomedullin to the right. The pD_{2} and E_{max} values were 7.02 ± 0.10 and 72.84 ± 3.5% in the absence of adrenomedullin_{22-52} and were 6.66 ± 0.12 and 58.08 ± 6.26% in the presence of adrenomedullin_{22-52} (10 μM), respectively. Incubation of the artery rings with CGRP_{8-37} (1 μM) also shifted the adrenomedullin concentration-response curve to the right (pD_{2}, 6.55 ± 0.15; E_{max}, 53.83 ± 6.78%) (Fig. 1A; Table 1). The maximal relaxation of mesenteric arterial strips by adrenomedullin was suppressed approximately 42 and 47% by adrenomedullin_{22-52} (10 μM) and CGRP_{8-37} (1 μM), respectively.

#### Effect of Inhibition of Nitric Oxide Synthesis on Adrenomedullin-Induced Vasodilation

To confirm the endothelial denudation and the lack of nitric oxide contribution to the adrenomedullin-induced vasorelaxation, concentration-dependent responses were developed both in the presence and absence of L-NAME (100 μM). L-NAME did not alter the adrenomedullin-induced concentration relaxation response in endothelium-denuded mesenteric artery rings. The pD_{2} and E_{max} values were 6.99 ± 0.11, 6.9 ± 0.19, 72.41 ± 4.24, and 68.18 ± 3.82%, respectively, in the presence and absence of L-NAME (Fig. 1B; Table 1).

#### Effect of Inhibition of Adenylate Cyclase or Protein Kinase A on Adrenomedullin-Induced Vasodilation

The involvement of cAMP-protein kinase A pathway in the adrenomedullin-induced endothelium-independent vasodilation in the mesenteric artery was assessed by inhibiting adenylate cyclase or protein kinase A using SQ22556 or Rp-cAMP, respectively. The adrenomedullin-induced concentration-dependent response curve was shifted to the right to a similar extent by both SQ22556 (pD_{2}, 6.66 ± 0.07%; E_{max}, 52.24 ± 9.8%) and Rp-cAMP (pD_{2}, 6.75 ± 0.08%; E_{max}, 57.02 ± 8.8%) compared with the controls in the absence of inhibitors (pD_{2} and E_{max} were 7.02 ± 0.10 and 72.84 ± 3.5%, respectively) (Fig. 2A; Table 1).

#### Adrenomedullin-Induced cAMP Generation in Mesenteric Artery

To confirm the generation of cAMP by adrenomedullin and the receptor mediation in this effect, we measured the basal and adrenomedullin-induced increases

### Table 1

<table>
<thead>
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<th>Treatment</th>
<th>n</th>
<th>pD2</th>
<th>Emax (% Relaxation)</th>
</tr>
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<tr>
<td>Control</td>
<td>8</td>
<td>7.03 ± 0.02</td>
<td>72.77 ± 3.56</td>
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<td>AM22–52 (10 μM)</td>
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<td>6.66 ± 0.12*</td>
<td>58.08 ± 6.26*</td>
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<td>6.55 ± 0.15*</td>
<td>53.83 ± 6.78*</td>
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<td>L-NAME (10 μM)</td>
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<td>6.90 ± 0.19</td>
<td>68.18 ± 8.32</td>
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<tr>
<td>SQ22556 (10 μM)</td>
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<td>6.66 ± 0.07*</td>
<td>52.24 ± 9.8*</td>
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<tr>
<td>Rp-cAMP (10 μM)</td>
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<td>6.75 ± 0.08*</td>
<td>57.02 ± 8.86*</td>
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<td>4-AP (1 mM)</td>
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<td>7.13 ± 0.09</td>
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<td>Glibenclamide (10 μM)</td>
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<td>7.18 ± 0.13</td>
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<td>BaCl2 (10 μM)</td>
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<td>79.63 ± 5.33</td>
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<td>Paxilline (1 μM)</td>
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<td>63.19 ± 6.93</td>
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<td>Iberiotoxin (100 nM)</td>
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<td>6.62 ± 0.06*</td>
<td>58.92 ± 3.89</td>
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<td>TEA (1 mM)</td>
<td>6</td>
<td>43.92 ± 6.15**</td>
<td></td>
</tr>
<tr>
<td>SQ22556 (10 μM) + TEA (1 mM)</td>
<td>4</td>
<td>45.47 ± 8.75**</td>
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<tr>
<td>Rp-cAMP (10 μM) + TEA (1 mM)</td>
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<td>45.98 ± 6.95**</td>
<td></td>
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<tr>
<td>80 mM KCl</td>
<td>4</td>
<td>49.87 ± 3.64**</td>
<td></td>
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</tbody>
</table>

TEA, tetraethylammonium.

*p ≤ 0.05, **p ≤ 0.01 compared to control, paired t test.

### Figure 1

Effect of adrenomedullin receptor antagonists adrenomedullin_{22-52} (10 μM) and CGRP_{8-37} (1 μM) (A) and inhibition of nitric oxide synthesis by L-NAME (100 μM) (B) on adrenomedullin-induced relaxation of endothelium-denuded mesenteric artery from day 18 pregnant rats. Values are mean ± S.E.M. of n observations.

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**TABLE 1**

Effect of different enzyme inhibitors, channel blockers, or receptor antagonist on AM-induced concentration-dependent relaxation of endothelium-denuded mesenteric artery from day 18 pregnant rats. Values are mean ± S.E.M. of n observations.

**Fig. 1.** Effect of adrenomedullin receptor antagonists adrenomedullin_{22-52} and CGRP_{8-37} (1 μM) (A) and inhibition of nitric oxide synthesis by L-NAME (100 μM) (B) on concentration-dependent relaxation induced by adrenomedullin in endothelium-denuded day 18 pregnant rat mesenteric artery precontracted by ED_{50} concentration of norepinephrine. Values are mean ± S.E.M.; n = figure in parentheses. Data were analyzed by two-way repeated measures ANOVA followed by Bonferroni’s post test. a, p ≤ 0.05 versus adrenomedullin_{22-52}; b, p ≤ 0.05 versus CGRP_{8-37}.
in the levels of cAMP in the presence and absence of adrenomedullin (100 μM) in the mesenteric artery. The basal CAMP levels were 0.38 ± 0.07 pmol/mg tissue, and adrenomedullin induced an increase in CAMP levels (2.00 ± 0.47 pmol/mg tissue). Adrenomedullin (100 μM) completely inhibited cAMP generated by adrenomedullin (0.3 μM) to 0.24 ± 0.04 pmol/mg tissue (Fig. 2B).

**Effect of Extracellular High Potassium (80 mM) on Adrenomedullin-Induced Vasodilation.** Depolarization by high potassium (80 mM) physiological saline solution produced a phasic contraction followed by a sustained tonic contraction. Concentration-dependent relaxation response was elicited by cumulative addition of adrenomedullin. The relaxation response to adrenomedullin was reduced in potassium-depolarized tissue (max, 70.66 ± 2.98%; n = 13) (Fig. 3A).

**Effect of Blockade of KATP, Kv, and Kir Channels on Adrenomedullin-Induced Vasorelaxation.** To elucidate the types of potassium channel involved in adrenomedullin-induced vasodilation, if any, we incubated the mesenteric artery with respective potassium channel blockers for 30 min, and the concentration-dependent response curve of adrenomedullin was constructed. Blockade of KATP, Kv, or Kir channels by glibenclamide (10 μM), 4-AP (1 mM), or BaCl2 (10 mM), respectively, did not alter the position of the concentration-response curve of adrenomedullin, indicating the lack of involvement of these potassium channels in adrenomedullin-induced vasodilation (Fig. 3, B, C, and D; Table 1).

**Effect of Blockade of Calcium-Activated Potassium Channel on Adrenomedullin-Induced Vasorelaxation.** The involvement of KCa channels in adrenomedullin-induced vasorelaxation was assessed by blocking them with tetraethylammonium (1 mM). Incubation of arterial rings with tetraethylammonium for 30 min inhibited the Emax of adrenomedullin from 72.84 ± 3.5 to 43.92 ± 6.15% (Fig. 4A). We then confirmed the involvement of KCa channels using iberiotoxin (100 nM), a selective and reversible inhibitor of high-conductance calcium-activated potassium channels, which reduced the Emax of adrenomedullin to 58.92 ± 3.89% from 73.17 ± 7.96% (Fig. 4B). In addition, paxilline (1 μM), another selective blocker of high-conductance calcium-activated (Maxi-K) potassium channels, shifted the adrenomedullin-induced concentration-response curve to the right with a pD2 of 6.75 ± 0.11% and Emax of 63.19 ± 6.93% (Fig. 4C). To assess the upstream dependence of KCa channels opening to cAMP action, adrenomedullin-induced concentration-response curve was developed in the presence of both SQ22536 (10 μM) or Rp-cAMP (10 μM) and tetraethylammonium (1 mM). The magnitude of shift of the concentration curve to the right was almost similar to the independent inhibition of either KCa channels or adenylyl cyclase or protein kinase A. There were no additive effects by simultaneous inhibition of adenylyl cyclase or protein kinase A and blockade of KCa channels on the inhibition of adrenomedullin-induced vasorelaxation (Fig. 4D; Table 1).

**Discussion**

The primary objective of this study was to investigate the mechanisms underlying the endothelium-independent relaxant responses to adrenomedullin in endothelium-denuded mesenteric arteries obtained from pregnant rats. The data from our studies showed that 1) adrenomedullin was able to relax the mesenteric artery, even in the absence of endothelium; 2) the adrenomedullin-induced relaxation was receptor-mediated as the receptor antagonist adrenomedullin22–52 or CGRP8–37 shifted the dose-response curve to the right; 3) inhibition of adenylyl cyclase or protein kinase A reduced the adrenomedullin-induced vasorelaxation to the same magnitude; 4) adrenomedullin increased the intracellular cAMP levels, which was blocked by adrenomedullin22–52; 5) 80 mM potassium reduced the adrenomedullin-induced relaxation,
whereas blockade of $K_{ATP}$, $K_V$, or $K_{IR}$ channels did not affect the adrenomedullin-induced vasorelaxation; and 6) blockade of $K_{Ca}$ inhibited the adrenomedullin-induced relaxation, whereas there was no additional inhibition by $K_{Ca}$ inhibitor when adenylate cyclase or protein kinase A was already inhibited. These data provide evidence for the involvement of cAMP-dependent protein kinase A and $K_{Ca}$ channels in mediating as the cellular pathways in the adrenomedullin-induced endothelium-independent relaxation of mesenteric artery from pregnant rats.

The distal portion of the secondary branches of mesenteric arteries (arterial diameter ~ 250 μm) from pregnant rats was used in this study. The mesenteric feed arteries and microcirculatory vessels have been reported as resistance vessels in freely moving rats (Fenger-Gron et al., 1995). It is generally accepted that arteries of a diameter up to 300 μm are true resistance vessels (Christensen and Mulvany, 2001). Hence, we considered the vessel strip used in this study as a resistance vessel. Adrenomedullin mediates its effects through receptors, which are heterodimeric complexes of the CL together with RAMP2 (AM1) or RAMP3 (AM2) (McLatchie et al., 1998). The receptor AM1 can be antagonized by the weak adrenomedullin peptide antagonist adrenomedullin$_{22-52}$, whereas the AM2 receptors can respond to both CGRP and adrenomedullin, which can be antagonized more potently by CGRP$_{8-37}$ compared with adrenomedullin$_{22-52}$ (Brain and Grant, 2004). In this study, the relaxant response to adrenomedullin at lower concentrations was inhibited by adrenomedullin$_{22-52}$, only while the relaxant responses at higher concentrations were reduced by both CGRP$_{8-37}$ and adrenomedullin$_{22-52}$. It is possible that the threshold response to adrenomedullin is mediated mainly through AM1 receptors, whereas the subsequent higher responses are mediated through both AM1 and AM2 receptors.

Vascular smooth muscle is known to relax in response to cAMP (Little et al., 1984), and it is reported that adrenomedullin has the ability to increase cAMP in human platelets (Kitamura et al., 1993). In this study, we found that inhibition of adenylate cyclase reduced the adrenomedullin-induced relaxation, indicating the involvement of cAMP. It is also supported by the increased cAMP levels in mesenteric arterial arcade after incubation with adrenomedullin. Previous studies have demonstrated that adrenomedullin caused increases in cAMP levels in cultured smooth muscle cells from the rat thoracic aorta (Eguchi et al., 1994; Ishizaka et al., 1994). This adrenomedullin-induced cAMP generation is receptor-mediated because adrenomedullin$_{22-52}$ (100 μM) was able to completely block the adrenomedullin-induced increases in cAMP levels. Inhibition of cAMP-dependent protein kinase A also reduced the adrenomedullin-induced vasorelaxation to a similar extent as that of inhibition of adenylate cyclase. This indicates a role of cAMP-protein kinase A

![Fig. 3. Effect of 80 mM K⁺ Cl (A) or inhibition of $K_{ATP}$ (B), $K_V$ (C), or $K_{IR}$ (D) channels on adrenomedullin-induced concentration-dependent relaxation of day 18 pregnant rat endothelium-denuded mesenteric artery precontracted by ED70 concentration of norepinephrine. Values are mean ± S.E.M.; $n$ = number in parentheses, two-way repeated measures ANOVA with Bonferroni’s post-test.](image-url)
pathway in the endothelium-independent mechanism of vascular relaxation caused by adrenomedullin.

Adenosine triphosphate-sensitive potassium channels are activated by protein kinase A in several arterial smooth muscle cells (Way et al., 1983). Adrenomedullin-induced relaxation was unaffected by glibenclamide at a concentration that blocks vascular K<sub>ATP</sub> channels (Quayle et al., 1994), indicating that K<sub>ATP</sub> channels are not involved. The lack of a role for K<sub>ATP</sub> channel is also reported in rat mesenteric artery (Champion et al., 2001), similar to the current study. In contrast, K<sub>ATP</sub> channels have been shown to mediate adrenomedullin-induced relaxation in dog coronary artery (Sabates et al., 1997). Inward rectifier potassium channels and KV channels are involved in the arterial relaxation caused by several vasodilators (Zhao et al., 1997; Huang et al., 2002). Concentrations of BaCl<sub>2</sub> (Robertson et al., 1996) or 4-AP (Yuan, 1995) that selectively block vascular K<sub>IR</sub> or KV channels, respectively, did not modulate the adrenomedullin-induced relaxation in mesenteric artery, indicating their lack of involvement. On the other hand, blockade of K<sub>Ca</sub> channels reduced the adrenomedullin-induced relaxation. We confirmed the involvement of K<sub>Ca</sub> channels by using iberiotoxin, a selective and reversible inhibitor of high-conductance K<sub>Ca</sub> channels and tetraethylammonium, at concentrations that inhibited single arterial BK channels (Langton et al., 1991). Paxilline, another selective blocker of high-conductance K<sub>Ca</sub> channels, also reduced the relaxant responses to adrenomedullin. These novel observations support the notion that adrenomedullin may activate K<sub>Ca</sub> channels and that the resultant membrane hyperpolarization would inhibit calcium influx via voltage-gated calcium channels. The shift of the concentration-response curve of adrenomedullin is similar in both independent blockade of K<sub>Ca</sub> channels or inhibition of adenylyl cyclase or protein kinase A and combined K<sub>Ca</sub> channel blockade and adenylyl cyclase or protein kinase A inhibition. Although there is no direct electrophysiological study using adrenomedullin, studies with CGRP, a peptide related to adrenomedullin, are reported with similar findings. In cultured smooth muscle cells from a porcine coronary artery, extracellular application of CGRP activated both K<sub>ATP</sub> and K<sub>Ca</sub> channels, only if the protein kinase A was not inhibited in cell-attached patch configuration (Miyoshi and Nakaya, 1995). In excised inside-out patches, application of cAMP or protein kinase A to the cytoplasmic side of the membrane activated the K<sub>Ca</sub> channel (Minami et al., 1993). Hence, it is more likely that cAMP-dependent protein kinase A plays a role as intracellular messenger pathway to adrenomedullin receptors and activates K<sub>Ca</sub> channels, which in

Fig. 4. Effect of inhibition of either K<sub>Ca</sub> channels alone by tetraethylamonium (1 mM) (A), iberiotoxin (100 nM) (B), paxilline (1 μM) (C), or K<sub>Ca</sub> (D) channels along with inhibition of adenylyl cyclase or protein kinase A [SQ22536 (10 μM) + tetraethylammonium (1 mM)] on adrenomedullin-induced concentration-dependent relaxation of endothelium-denuded day 18 pregnant rat mesenteric artery precontracted with ED<sub>50</sub> concentration of norepinephrine. Values are mean ± S.E.M. n = values in parentheses. Two-way repeated measures ANOVA followed by Bonferroni’s post-test. *, p ≤ 0.05; **, p ≤ 0.01 versus control.
turn reduces the intracellular calcium and causes the relaxation of the artery. Previously, our laboratory reported that infusion of pregnant rats with CGRP\textsubscript{8-37} increased blood pressure and fetal mortality and retarded fetal growth (Gangula et al., 2002). Because CGRP\textsubscript{8-37} can also block AM\textsubscript{2} receptors (Brain and Grant, 2004), it is possible that endogenous adrenomedullin plays a significant role in the gestational vascular adaptation.

In conclusion, the present study demonstrates a significant role for KC\textsubscript{a} channels in adrenomedullin-induced endothelium-independent relaxation in the isolated mesenteric artery from pregnant rats. Adrenomedullin may activate KC\textsubscript{a} channels but not other potassium channels, mostly through a cAMP-dependent protein kinase A-dependent cellular mechanism. The relaxation effects of adrenomedullin on mesenteric artery, a resistance vessel from pregnant rats, may contribute to the marked vascular adaptations during pregnancy.

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References


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