AM reverses pressor response to ET-1 independently of NO in rat coronary circulation

PIETARI KINNU nen, JARKKO PIUHOLA, HEIKKI RUSKOAH O, and ISTVÁN SZOKODI. AM reverses pressor response to ET-1 independently of NO in rat coronary circulation. Am J Physiol Heart Circ Physiol 281: H1178–H1183, 2001.—Endothelin-1 (ET-1) elicits a vasoconstrictor response via ETA receptors, whereas simultaneous activation of ETB receptors triggers the release of nitric oxide (NO), which may limit the constrictor effect of ET-1. Recently, stimulation of ETB receptors has been shown to increase the secretion of adrenomedullin (AM), a newly identified vasorelaxing peptide. The present study was designed to see whether AM can oppose the vasoconstrictor response to ET-1. In the isolated perfused paced rat heart preparation, infusion of ET-1 at concentrations of 1 nmol/l for 30 min induced a significant coronary vasoconstriction, whereas it had no effect on perfusion pressure at a dose of 0.08 nmol/l. Nω-nitro-L-arginine methyl ester (L-NAME; 300 μmol/l), a potent inhibitor of NO synthase (NOS), did not change the perfusion pressure when added alone to the perfusion fluid but it unmasked the constrictor effect of ET-1 at both concentrations. In the presence of L-NAME, AM (0.03 to 1 nmol/l) markedly reversed the pressor response to ET-1 at both concentrations. Administration of AM (0.03 and 1 nmol/l) alone resulted in a dose-dependent decrease in perfusion pressure, which was not modified in the presence of L-NAME. In conclusion, the coronary vasoconstrictor response to ET-1 is markedly augmented in the presence of a NOS inhibitor. This constrictor response is substantially reversed by AM. Our results indicate that AM may serve as a paracrine modulator of ET-1-induced vasoconstriction independently of the NO pathway. 

The coronary vascular endothelium modulates vascular tone through the release of vasodilating and vasoconstricting substances, among them nitric oxide (NO) and endothelin-1 (ET-1). Endothelial NO synthase (eNOS), the major NOS isoform expressed in endothelial cells, constitutively catalyzes the formation of NO from the amino acid L-arginine (44). NO is generally considered to be the primary endothelium-derived relaxing factor regulating vascular resistance (10). ET-1, on the other hand, is an endothelium-derived peptide (46) possessing a potent and long-lasting vasoconstrictor effect (28). The actions of ET-1 are mediated by two subtypes of G-protein-coupled heptahelical receptors named ET_A and the ET_B receptors (1, 31). ET-1 produces coronary vasoconstriction at pathophysiological concentrations predominantly via binding to ET_A receptors located on vascular smooth muscle cells (4). However, simultaneous activation of ET_B receptors on endothelial cells triggers the release of NO (12, 43), which in turn could limit the constrictor effect of ET-1. Accordingly, removal of this negative feedback by the blockade of eNOS has been shown to augment the effect of ET-1 on coronary vascular tone (21, 45).

In addition to NO, there are several locally produced vasodilator substances that may also modulate the effect of ET-1. However, little information is available on which vasoactive factors may play such a role in situations associated with a decreased bioavailability of endothelium-derived NO. A growing body of evidence (14, 32) suggests a potential role for adrenomedullin (AM) in the regulation of vascular tone. When infused intravenously, AM induces a potent and long-lasting hypotensive effect (5, 16). Furthermore, the coronary relaxant effect of AM has been shown to be comparable to that of calcitonin gene-related peptide (CGRP) (8), the most potent vasodilator known so far (2). The presence of specific AM receptors on vascular smooth muscle cells (7), combined with findings of synthesis and secretion of AM in vascular endothelial cells (13, 35) and smooth muscle cells (36), suggest that AM may act predominantly in a paracrine-autocrine way. ET-1 was reported to enhance the production of AM in cultured vascular smooth muscle cells (37). Moreover, Jougasaki et al. (15) recently demonstrated that the stimulation of ET_B receptors increases the secretion of AM in vascular endothelial cells. However, no information is available on the functional interaction between AM and ET-1. Therefore, the objective of the present study was to examine whether AM can oppose the coronary vasoconstriction induced by ET-1. We were particularly interested in the interplay of AM and ET-1 under the conditions of pharmacological inhibition of NO synthesis, because both NO-
MATERIALS AND METHODS

Drugs. The drugs used in the study were ET-1 (Peninsula Laboratories), rat AM-(1–50) (Phoenix Pharmaceuticals), and Nω-nitro-L-arginine methyl ester (L-NAME, Sigma).

Animals. Male Sprague-Dawley rats (n = 94, weighing 241 ± 2 g) from the colony of the Center of Experimental Animals at the University of Oulu were used. The rats were housed in plastic cages in a room with a controlled humidity of 40% and a temperature of 22°C. A 12:12-h light-dark environmental light cycle was maintained. The experimental design was approved by the Animal Use and Care Committee of the University of Oulu. The study protocol conforms to the European convention for the protection of vertebrate animals used for experimental and other scientific purposes.

Isolated perfused rat heart preparation. The isolated perfused rat heart preparation used in this study was similar to that described previously (22, 38, 39). Briefly, rats were decapitated and hearts were quickly removed and arranged for retrograde perfusion by the Langendorff technique. The hearts were perfused with a modified Krebs-Henseleit bicarbonate buffer (pH 7.40), equilibrated with 95% O2-5% CO2 at 37°C. The buffer was composed of (in mmol/l) 113.8 NaCl, 22.0 NaHCO3, 4.7 KCl, 1.2 KH2PO4, 1.1 MgSO4, 2.5 CaCl2, and 11.0 glucose.

An initial preload stretch of 2 g was set by connecting a force displacement transducer (model FT03, Grass Instruments) to the apex of the heart. The heart rate was counted from contractions by the tachograph (Grass) and was then increased 15–20% above the spontaneous beating frequency from contractions by the tachograph (Grass) and was then increased 15–20% above the spontaneous beating frequency with the use of a stimulator (11 V, 0.5 ms; model S88, Grass) to avoid any secondary effects caused by changes in heart rate. Variations in perfusion pressure arising from changes in coronary vascular resistance were measured with the use of a pressure transducer (model MP-15, Micron Instruments) situated on a side arm of the aortic cannula. All recordings were made with the use of a polygraph (model 7DA, Grass). Each experiment was started by perfusion of the hearts for 60 min (equilibration period) at a flow rate of 7 ml/min with a peristaltic pump (model 312, Minipuls 3). To diminish the basal NO synthesis, which is regulated by the level of vascular shear stress (20), the flow rate was then decreased to the final experimental rate 5.5 ml/min, including the flow rate of the infusion cannula, as described previously (22, 38, 39).

Experimental design. A 10-min control period was followed by the addition of vehicle or drugs into the aortic perfusion cannula as a continuous infusion via an infusion pump (Sky electronics, Secan PSA 55) at a rate of 0.5 ml/min for 30 min. All hearts were used for one experiment only and the study was conducted in a controlled and randomized manner, i.e., vehicle and substances were run concomitantly and randomly. Vehicle, AM, and ET-1 was infused alone and in combination with L-NAME (300 μmol/l), a NOS inhibitor, into the coronary circulation. The concentration of L-NAME is known to be effective in the Langendorff preparation (P. Taskinen, O. Vuolteenaho, and H. Ruskoaho (unpublished data) and Pabla and Curtis (27)).

Statistics. Results are presented as means ± SE. The data were analyzed with two-way analysis of variance for repeated measurements. The statistical differences between two groups for one parameter were determined with Student's t-test. Differences were considered statistically significant at the level of P < 0.05.

RESULTS

Effects of ET-1 and AM on coronary vascular tone. The influence of various drug infusions on coronary perfusion pressure of the isolated rat heart are presented in Table 1. The preparation was stable throughout the experiments. Infusion of vehicle alone had no effect on the basal perfusion pressure (Fig. 1). Administration of ET-1 at a concentration of 0.08 nmol/l had no effect on vascular tone (Fig. 1A), but produced a significant coronary vasoconstrictor effect at 1 nmol/l (P < 0.001 vs. vehicle by two-way ANOVA for drug and time interaction, Fig. 1B). Despite the near-maximum dilatation of the coronary arteries induced by the relatively low coronary flow rate, infusion of AM (0.03 and 1 nmol/l) resulted in a dose-dependent decrease in perfusion pressure (Table 1 and Fig. 1).

Effect of inhibition of NO synthesis on ET-1 and AM-induced vascular responses. When L-NAME (300 μmol/l), an inhibitor of NOS, was infused alone into the

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Table 1. Changes in isolated rat coronary perfusion pressure in the presence or absence of NO synthase inhibitor L-NAME

<table>
<thead>
<tr>
<th>Group</th>
<th>Perfusion Pressure Without L-NAME, mmHg</th>
<th>Perfusion Pressure With L-NAME, mmHg</th>
<th>P vs. without L-NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>30 min</td>
<td>Δ%</td>
</tr>
<tr>
<td>Vehicle</td>
<td>32.1 ± 0.9</td>
<td>32.8 ± 1.0</td>
<td>2.0 ± 1.0</td>
</tr>
<tr>
<td>ET-1 (0.08 nmol/l)</td>
<td>30.2 ± 1.1</td>
<td>30.3 ± 1.1</td>
<td>0.5 ± 0.5</td>
</tr>
<tr>
<td>AM (0.03 nmol/l)</td>
<td>30.9 ± 0.9</td>
<td>29.2 ± 1.0</td>
<td>−5.7 ± 2.0</td>
</tr>
<tr>
<td>ET-1 (0.08 nmol/l) + AM</td>
<td>27.9 ± 1.8</td>
<td>28.6 ± 1.8</td>
<td>−4.2 ± 1.9</td>
</tr>
<tr>
<td>ET-1 (1 nmol/l)</td>
<td>31.4 ± 0.8</td>
<td>37.3 ± 2.0</td>
<td>19.1 ± 7.7</td>
</tr>
<tr>
<td>AM (1 nmol/l)</td>
<td>32.9 ± 1.7</td>
<td>28.4 ± 1.2</td>
<td>−13.4 ± 1.3ab</td>
</tr>
<tr>
<td>ET-1 (1 nmol/l) + AM</td>
<td>33.1 ± 1.0</td>
<td>35.2 ± 1.3</td>
<td>6.5 ± 3.7</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. L-NAME, Nω-nitro-L-arginine methyl ester; NO, nitric oxide; ET-1, endothelin-1; AM, adrenomedullin. Δ%, percentage of change in perfusion pressure from baseline to the end of the infusion. Perfusion pressure is reported at baseline and after 30-min infusion. *P < 0.001 vs. vehicle; †P = 0.01 vs. AM 0.03 nmol/l; ‡P < 0.001 vs. L-NAME alone; §P < 0.05 vs. ET-1 0.08 nmol/l with L-NAME; ‡P < 0.05 vs. ET-1 1 nmol/l with L-NAME by Student’s t-test. Increase in perfusion pressure induced by 1 nmol/l ET-1 without L-NAME was significant only when assessed as a function of time by two-way analysis of variance.
number of experiments and baseline values are presented in Table 1. Separate experiments that were run on different isolated rat hearts. Note the different scale of pressure change between A and B.

**Effect of AM on ET-1-induced coronary vasoconstriction.** In the presence of l-NAME, AM at a concentration of 1 nmol/l markedly reversed the pressor response to 1 nmol/l ET-1 (Table 1, Fig. 1B). Similarly, under the blockade of NO synthesis, the vasoconstrictor effect of ET-1 at 0.08 nmol/l was significantly attenuated by AM at 0.03 nmol/l (Table 1, Fig. 1A), further supporting the hypothesis that AM could exert its vasodilator effect independently of the NO pathway.

**Discussion**

The present results show for the first time that AM, a vasorelaxant peptide, potently attenuates the coronary vasoconstrictor response to ET-1 in vitro. Previous investigations (9, 11, 26, 34) have suggested that NO release is involved at least in part in the mechanisms of AM-induced vasodilation in various vascular beds. In contrast, our results suggest that AM can exert a profound coronary vasodilator effect under the blockade of NO synthesis. The interplay of ET-1 and AM has been previously studied mainly at the level of the synthesis and secretion of the peptides (15, 17, 37). The current findings extend these previous observations and supports the concept that AM may function as an endogenous modulator of ET-1-induced vasoconstriction independently of the l-arginine-NO pathway.

It has been reported (4) that administration of ET-1 in vivo at low doses mimicking pathophysiological concentrations induces a coronary constrictor effect predominantly via ETA receptors. However, simultaneous activation of ETB receptors, triggering the release of NO from endothelial cells (12, 43), may limit the constrictor effect of ET-1. Accordingly, removal of this negative feedback by the blockade of NO formation has been shown to augment the effect of ET-1 on vascular tone (21, 45). In agreement with the previous findings, the increase in perfusion pressure in response to ET-1 was markedly augmented in the presence of a NOS inhibitor also under our experimental conditions. Whether other vasodilator pathways may also counteract the constrictor effect of ET-1 is a fundamental question to address, especially in situations associated with a decreased bioavailability of endothelium-derived NO.

AM, as one of the most potent endogenous vasodilators (14, 32), is a particularly attractive candidate for such a role. Earlier studies have shown that AM is actively synthesized and secreted in endothelial cells (13, 35) and vascular smooth muscle cells (36). A cross-talk between ET-1 and AM has been suggested by the demonstration that ET-1 enhances the production of AM in cultured vascular smooth muscle cells (37). Jougasaki et al. (15) recently showed that the stimulation of ETB receptors increases the secretion of AM in coronary circulation, the perfusion pressure remained constant (Table 1 and Fig. 1), indicating that the impact of basal NO production on the coronary vascular tone is minimal under our experimental conditions, as described previously in vivo (4). However, inhibition of NO synthesis unmasked the constrictor effect of ET-1 at both concentrations (Table 1 and Fig. 1), showing that endogenous NO could almost maximally counteract the pressor response to ET-1. In fact, there was a dose-dependent increase in perfusion pressure by ET-1 in the presence of l-NAME (Table 1). When AM was infused into the coronary circulation in combination with l-NAME, it reduced the perfusion pressure to a similar extent as observed in the absence of inhibition of NOS (Table 1), indicating that NO does not appear to mediate AM-induced vasodilatation in rat coronary arteries under our experimental conditions.

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vascular endothelial cells, suggesting that the peptide (in a fashion similar to NO) may function to buffer the vasoconstrictor effect of ET-1. In agreement with this hypothesis, our results show that AM markedly attenuates the coronary vasoconstriction induced by ET-1. The existence of a paracrine-autocrine regulatory loop between ET-1 and AM is further supported by our finding showing that administration of ET-1 significantly increases the release of AM into the coronary effluent of the perfused rat heart as measured by a specific radioimmunoassay. Infusion of ET-1 (1 nmol/l) for 120 min increased perfusate immunoreactive AM (ir-AM) levels by 1.6-fold (from 78 ± 12 to 127 ± 8 fmol/l, n = 6; P < 0.05), whereas infusion of vehicle alone had no effect on ir-AM levels (n = 6, P = not significant). Moreover, the increase in perfusate ir-AM levels presumably reflects merely the spillover of a tiny fraction of that produced locally. Taken together, these data indicate an intimate, specific relationship between ET-1 and AM.

Previously, AM has been reported to trigger NO synthesis via Ca²⁺-dependent activation of eNOS in endothelial cells (33). Furthermore, the stimulation of NO release appears to contribute to the vasorelaxant effect of AM in various rat vascular preparations, including renal (11), pulmonary (26), and hindlimb (9) vascular beds. Of special importance is our finding that AM could reverse the coronary vasoconstriction induced by ET-1 under the blockade of NO synthesis suggesting that AM may represent an alternative pathway distinct from NO to counteract the pressor response to ET-1 in the rat coronary vasculature. In previous studies (47) in porcine coronary artery rings, denudation of the endothelium did not modulate the relaxant effect of AM, whereas Terata et al. (41) recently reported that in human coronary arterioles AM elicited vasodilation in part through production of NO and in part through activation of K⁺ channels. In addition to the different experimental conditions, the differences in regulation of eNOS activity between the species may explain the discrepancy between these studies. The cross-talk between ET-1 and AM in vascular smooth muscle cells may occur at the level of the regulation of intracellular [Ca²⁺] because ET-1 has been reported to increase (40) and AM to decrease (7, 19) Ca²⁺ levels via stimulation of phospholipase C or adenylate cyclase, respectively. Alternatively, ET-1 and AM may interact at ATP-sensitive K⁺ channels of smooth muscle cells, because it has been demonstrated that ET-1 can block (24) and AM can activate (29) these channels. Whether endogenous AM can act as a buffer for the coronary constrictor effect of ET-1 needs to be investigated further. However, there are currently no selective antagonists for AM receptors available. Under our experimental conditions, the truncated peptides AM26–52 and CGHRP8–37 failed to antagonize the coronary vasodilator effect of AM (data not shown).

The synthesis of ET-1 is regulated by numerous stimuli including ischemia (3) and hypoxia (18), and the enhanced levels of ET-1 that occur during pathophysiological states (e.g., myocardial ischemia) may contribute to the further exaggeration of coronary constriction (28). ET-1 can also augment its own gene expression through ET₁ receptors in endothelial cells (30), providing a unique positive feedback mechanism. Furthermore, the constrictor effect of ET-1 is likely to be enhanced by a simultaneous impairment of NO-dependent relaxation due to decreased bioavailability of NO in various pathophysiological conditions including atherosclerosis (23) and prolonged hypoxia (42). In contrast, AM synthesis and secretion has been reported to be markedly augmented in cultured endothelial cells by hypoxia (25) and increased oxidative stress (6). Because AM has been shown (17) to suppress the production of ET-1 in cultured endothelial cells and because AM can attenuate the coronary constrictor effect of ET-1 as shown in this study, it is tempting to speculate that AM may act against the vasoconstriction maintained by ET-1. These observations suggest a possible therapeutic role for AM or its analogues in the management of coronary syndromes.

In conclusion, we show that the coronary vasoconstrictor response to ET-1 is markedly augmented by pharmacological inhibition of endogenous NO formation and that this enhanced constrictor effect is substantially reversed by AM. Given that the effect of AM is independent of the l-arginine-NO pathway, the coronary vasodilator response to AM may remain intact in pathophysiological states such as atherosclerosis associated with an impaired NO synthesis. These findings are consistent with the hypothesis that AM may play a compensatory role against excessive coronary vasoconstriction induced by ET-1 in pathophysiological states.

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