Intermedin (IMD/AM2) dilates the pig coronary vascular bed through release of nitric oxide

İntermedin (IMD/AM2), nitrik oksit salımı yoluyla domuz koroner vasküler yatağında dilatasyon sağlamaktadır

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Background: This study investigated the effects of intermedin/adrenomedullin-2 (IMD/AM2), an endogenous agonist for calcitonin-like calcitonin receptors, on coronary and systemic hemodynamics.

Methods: Ultrasonic transit time flow probes were placed around the left anterior descending (LAD) artery in the anesthetized, open-chest pig (n=6). A catheter was placed into the proximal LAD. Intracoronary arterial bolus injections of IMD, adrenomedullin (hADM13-52) and calcitonin gene related peptide (CGRP) (1, 3, 10 μg) were performed and the changes in the velocity of coronary blood flows were continuously recorded.

Results: Intracoronary artery bolus injections of IMD, hADM13-52 and CGRP increased coronary blood flow in a dose-dependent manner. At the studied doses, IMD was more potent than CGRP and hADM13-52 and did not alter systemic arterial pressure, cardiac output and cardiac index. Intracoronary artery injection of NG-Nitro-L-arginine-methyl ester (L-NAME) significantly decreased the coronary vasodilator response (CVR) to IMD.

Conclusion: The present data suggest that IMD possesses marked vasodilator activity in the pig coronary vascular bed. The present data further suggest that IMD acts on a receptor in the coronary vascular bed that is coupled to endothelial nitric oxide release. The degree of the CVR to IMD may serve as functional marker for the integrity of endothelial cells in resistance segments of the coronary circulation

Key words: Coronary vascular bed; intermedin; nitric oxide; vasodilatation.

Amaç: Bu çalışmada kalsitonin benzeri kalsitonin reseptörlerinin endojen bir agonisti olan intermedin/adrenomedüllin-2'nin (IMD/AM2) koroner ve sistemik hemodinami üzerindeki etkileri araştırıldı.

Çalışma planı: Aneztezi altındaki göğsü açık domuzların (n=6) sol ön inen koroner arterleri (LAD) etrafına ultrasonik geçiş süresi akım probları yerleştirildi. Proksimal LAD içerisine bir kateter yerleştirildi. İntrakoroner bolüs IMD, adrenomedüllin (hADM13-52) ve kalsitonin geniyle ilişkili peptid (CGRP) (1, 3, 10 μg) enjeksiyonları uygulandı ve koroner akım hızlarında meydana gelen değişiklikler sürekli olarak kayıt edildi.

Bulgular: İntrakoroner bolüs IMD, hADM13-52 ve CGRP enjeksiyonları koroner kan akımlarını doza bağımlı şekilde artırdı. Araştırılan dozlarda IMD, CGRP ve hADM13-52'den daha güçlü bir etkiye sahip idi ve sistemik arteriyel basınçta, kalp debisinde ve kardiyak indekste değişikliğe neden olmadı. İntrakoroner NG-Nitro-L-arginine-methyl ester (L-NAME) enjeksiyonu IMD'ye gelişen koroner vazodilatör yanıtı (CVR) anlamlı derecede azalttı.

Sonuç: Bu çalışmanın verileri IMD'nin domuz koroner vasküler yatağı üzerine belirgin bir vazodilatör etkiye sahip olduğuna işaret etmektedir. Ayrıca bu veriler IMD'nin koroner vasküler yatakta endoteliyal nitrik oksit salımı ile ilişkili bir reseptör üzerinde etki gösterdiğine işaret etmektedir. IMD'ye gelişen CVR'nin derecesi koroner dolaşımdaki direnç bölümlerinde bulunan endoteliyal hücrelerin bütünlüğünün işlevsel bir göstergesi olarak kullanılabilir.

Anahtar sözcükler: Koroner vasküler yatak; intermedin; nitrik oksit; vazodilatasyon.

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Since the discovery of calcitonin in the 1960s, many bioactive peptides such as calcitonin gene-related peptide (CGRP), adrenomedullin (ADM), and amylin were discovered to be structurally related to each other and therefore grouped into the CGRP superfamily.[1] This group of peptide hormones acts on diverse organs and tissues and regulates body homeostasis. Adrenomedullin and CGRP are important endocrine and neurocrine integrators of homeostasis in the cardiovascular, renal and respiratory systems, whereas amylin is essential for optimal glucose metabolism.[2-4] Recently Roh et al.[5] identified a novel calcitonin/CGRP family peptide, intermedin (IMD) from the genomes of human and other vertebrates. Human intermedin encodes a prepropeptide of 148 amino acids, with a signal peptide for secretion at the N terminus.[5] Intermedin is a 47 amino acid peptide formed by enzymatic degradation of preprointermedin. Initially isolated from the puffer fish, the IMD sequence is conserved across species including human, rat and mouse.[5] Intermedin has been reported to be expressed in the kidney, lung, thymus, gastrointestinal tract, submaxillary gland and brain by using real time-polymerase chain reaction (RT-PCR).[5,6] Intracerebrovascular administration of IMD promotes anorexia, water restriction, release of prolactin, oxytocin, vasopressin and adrenocorticotropin as well as inhibition of growth hormone release.[7-11] Peripheral administration, similar to intracerebrovascular administration, promotes anorexia. However it also promotes gastoparesis, oliguria, diuresis and antinatriuresis.[7]

Systemic and regional vascular responses to IMD have been reported in the conscious and anesthetized mouse and rat.[6,10-12] Intracerebrovascular administration of IMD increases systemic arterial pressure (SAP), whereas intravenous (i.v) administration of IMD decreases SAP.[6,10,13] Intrarenal infusion of IMD increases renal blood flow and decreases renal vascular resistance in vivo[7,9] whereas IMD does not relax porcine renal arterial conductance vessels. Moreover, IMD reduces myocardial injury in the ischemia-reperfused rat heart in vitro and relaxes isolated porcine coronary arterial rings.[14-16] Although studied in isolated porcine coronary artery (in vitro), the effects of IMD on the coronary vascular bed in vivo are unknown.

The purpose of the present study was undertaken to investigate the effects of IMD, an endogenous agonist for calcitonin-like calcitonin receptors (CRLR), on the pig coronary hemodynamics in vivo.

MATERIALS AND METHODS

The experiment was performed in compliance with the "Principles of Laboratory Animal Care" formulated by the National Institutes of Health (National Institutes of Health publication no. 85-23, revised 1996). The Ethics Committee for Animal Care, established in our institute, approved the experiment and animal care protocol.

Materials

N^G-Nitro-L-arginine-methyl ester (L-NAME) was obtained from Sigma Chemical Co (St. Luis, MO, USA). Intermedin, $hADM_{13-52}$, and CGRP were purchased from Bachem (Torrance, CA, USA). Peptides were initially dissolved in distilled water to make a stock solution and were subsequently with normal saline to working solution on the day of use.

Open-chest pig experiment protocol

Porcine subjects of both sexes (n=6), mean weights 62±16 kg (ranging from 49-83) were premedicated with ketamine (20 mg/kg intramuscularly; im), midazolam (0.1 mg/kg im), and atropine (0.25 mg im) and placed in the supine position. Anesthesia was induced with midazolam, 0.1 mg/kg, plus sufentanil, 0.5 μg/kg, and was maintained with intravenous infusions of sufentanil, 0.5 μg/kg/h, and midazolam, 0.15 mg/kg/h. Muscle paralysis was achieved with vecuronium bromide (1 mg/kg), and was maintained with an infusion of vecuronium at 2 mg/kg/h. The lungs were mechanically ventilated via a No. 9 cuffed endotracheal tube (Kendall Curity Tracheal Tube, Tyco Healthcare, Switzerland) with a Servo ventilator 900 C (Siemens, Elema, Sweden) initially set to deliver forced inspiratory oxygen (F_iO_2) of 0.4, tidal volume between 12 and 15 mL/kg, and respiratory rate adjusted to maintain partial pressure of carbon dioxide in arterial blood ($PaCO_2$) in the range of 35 to 40 mmHg. Positive end-expiratory pressure of 5 cmH_2O was used to prevent atelectasis. Sevoflurane was administered with a vaporizer adapted to the ventilator. Inspired and expired fractions of oxygen, carbon dioxide, and sevoflurane were measured with an infrared spectrophotometer (Ultima II; Datex, Helsinki, Finland; Fig. 1a-c).

Sodium chloride (0.9% at 10 mL/kg/h) was infused into the left internal jugular vein during surgery. Temperature was maintained at 38 °C to 39 °C with an electrical heating pad. An invasive arterial pressure monitoring line catheter was placed into the right common iliac artery for systemic arterial blood pressure and arterial blood sampling. A balloon-tipped flow-directed pulmonary artery catheter (Swan Ganz CCO/VIP; Edwards Lifesciences LLC, Irvine, CA, USA) was inserted through the right internal jugular vein and positioned under pressure control in a branch of the pulmonary artery for measurement of mean pulmonary arterial pressure (MPAP), central venous pressure (CVP), pulmonary capillary wedge pressure (PCWP), cardiac output (CO), cardiac index (CI), left

ventricle end diastolic pressure (LVEDP), and systemic vascular resistance (SVR) index.

After exposure with a midline sternotomy, the heart was suspended in a pericardial cradle. Ultrasonic transit time flow probes were placed around the distal part the left anterior descending (LAD) artery. A catheter was placed into the proximal part of the LAD. Intracoronary arterial bolus (IAB) injections of IMD (1, 3, 10 μg), $hADM_{13-52}$ and CGRP were performed and change in coronary blood flows continuously measured and recorded. Changes in the coronary blood flows were measured by ultrasonic flow device (Transonic systems Inc., NY, USA) as mL/min.

Between each injection, time was allowed to reestablish baseline coronary artery blood flow. In each pig, IMD, $hADM_{13-52}$, and CGRP dosages were bolus injected in random sequence into the LAD coronary artery before and after intracoronary artery infusion of L-NAME.

Changes were recorded by a camcorder continuously during the whole experiment period. After the experimental period data were extracted from the recorded DVDs.

Statistical analysis

The relaxant responses were expressed as a percentage of relaxation. Results are expressed as mean ± SEM and *n* represents the number of animals. Data were analyzed by one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls post hoc test. A *p*-value of less than 0.05 was considered statistically significant.

RESULTS

As displayed in Figures 1a, 1b and 1c, random sequence, intracoronary artery bolus injections of 1, 3, 10 mcg of IMD, $hADM_{13-52}$ and CGRP induced marked, dose-dependent increases in coronary blood flows that peaked within seconds of administration and lasted less than 20 seconds before returning to the baseline levels. None of these agents ever induced significant alterations in heart rate, systemic mean arterial pressure, cardiac output, left ventricle end-diastolic pressure, SVR and resistance index (data not shown). This observation strongly suggest that each of these agents was active at the point of administration and that the coronary artery blood flow increases were likely due to decreases in coronary vascular resistance. When compared to $hADM_{13-52}$ and CGRP under similar experimental conditions IMD was more active at the given doses of other agents.

In order to determine if the coronary vasodilator response to IMD, $hADM_{13-52}$ and CGRP is mediated by nitric oxide (NO), the effects of L-NAME on the coronary vascular responses to IMD, $hADM_{13-52}$, and CGRP were investigated in additional series of experiments in the same animals and results of these studies are illustrated in figures 1a-c. When compared to the coronary vasodilator responses to IMD in control experiments, increases in coronary flow in response to intracoronary injection of IMD after premedication with L-NAME were significantly reduced ($p<0.05$; Fig 1a). L-NAME pretreatment also inhibited the increase in

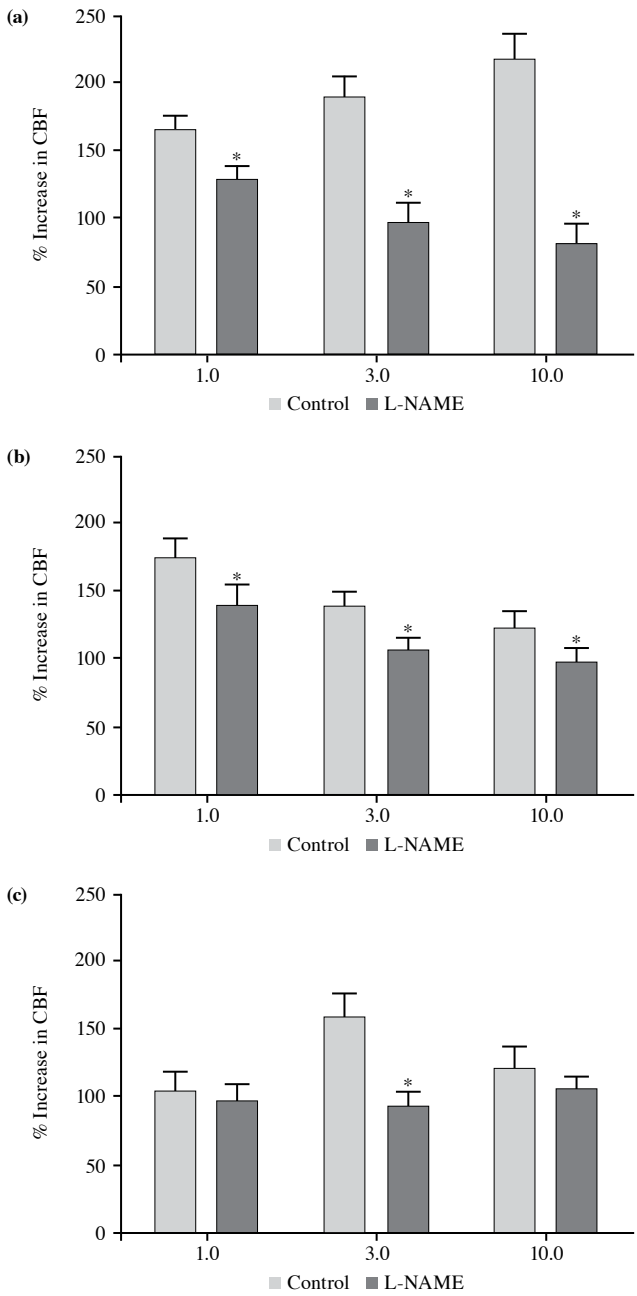

Fig. 1. Percent changes in coronary blood flow following the administration of **(a)** intermedin, **(b)** adrenomedullin and **(c)** calcitonin gene related peptide [n=6 (1.0, 3.0, 10.0 μg)]. *: $p<0.05$; CBF: Coronary blood flow; L-NAME: Nitro-L-Arginine Methyl Ester.

coronary blood flow in response to acetylcholine indicating L-NAME inhibited receptor dependent release of NO in the coronary artery.

DISCUSSION

Data from the present study indicate that the marked increases in coronary artery blood flow observed in response to direct injections of IMD are mediated through concomitant reductions in coronary vascular resistance and not through changes in heart rate, systemic mean arterial pressure, cardiac output and left ventricle end-diastolic volume. Coronary vasodilator response to IMD was inhibited by L-NAME suggesting that release of NO contributes to the coronary vasodilator response to IMD. The observed effect on regional (e.g. coronary) blood flow is consistent with several previously published studies of the effects of IMD on other regional vascular beds. Sabates et al.[16] demonstrated in a pig model that direct coronary artery injections of AMD produced dose-dependent increases in coronary blood flow. Lippton et al.[15] have demonstrated that direct intrapulmonary arterial AMD infusions produced marked vasodilatation by using a cat model in which a pulmonary lobar segment is placed under conditions of constant perfusion. Kandilci et al.[17] also demonstrated that IMD dilates the pulmonary vascular bed of the rat under conditions of actively increased pulmonary vasoconstrictor tone. In another study Burak Kandilci et al.[18] reported that rIMD possesses a concentration-dependent vasorelaxant effect on the preconstricted rat pulmonary artery and activates all types of CRLR receptors in a non-specific manner. Data from this study further suggests that release of NO from endothelium, the activation of protein kinase G and then calcium-activated potassium channels (BK_{Ca}) is the major pathway involved in the pulmonary vasorelaxant response to rIMD. This finding is consistent with the actions of adrenomedullin and CGRP, which are known to exert NO dependent vasodilator actions in the rat aorta, pulmonary artery, pulmonary vascular bed and kidney.[19] As further intracellular mechanisms proposed, IMD augments cardiomyocyte contractile function via both protein kinase A and protein kinase C dependent pathways.[20] Finally, a recent publication has reported IMD induced vasorelaxation in the mesenteric artery has been coupled with receptor dependent nitric oxide formation. Hence, in the same study IMD relaxation is cGMP and cAMP mediated with subsequent activation of potassium channels.[21]

Jia et al.[22] suggested that the intermedin receptor system was up-regulated in isoproterenol-induced myocardial ischemic injury and IMD_{1-53} might play a pivotal cardioprotective role in such injury. Dong et al.[20] suggested that IMD acutely augments cardiomyocyte contractile function through, at least in part, a protein kinase C- and protein kinase A-dependent mechanism. Pan et al.[23] showed that perfusion of isolated rat hearts in vitro with IMD8-47 (10(-8) and 10(-7) mol/L) resulted in lower left ventricle systolic pressure (LVSP), by 40 and 56% ($p<0.01$); lower +LVdp/dt (max), by 33 and 47% ($p<0.01$); lower -LVdp/dt (max), by 25 and 39% ($p<0.01$); but higher coronary perfusion flow, by 25% ($p<0.05$) and 33% ($p<0.01$), respectively, than controls. In this study although they perfused the rat hearts intravenously, they indicated that IMD resulted higher coronary perfusion flows. From this point of view, we can indicate that this is the first in vivo study demonstrating the effects of IMD on coronary vascular bed through direct injection. The present study suggests that all given peptides increased the coronary blood flow in a dose-dependent manner, but IMD caused the most potent increase in coronary blood flow.

In conclusion, as a conclusion, this study suggests that IMD possesses marked vasodilator activity in the pig coronary vascular bed. Furthermore the present data suggests the CVR to IMD in the pig is mediated by release of nitric oxide from endothelial CRLR. The degree of the CVR to IMD may serve as functional marker for the integrity of endothelial cells in resistance segments of the coronary circulation.

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Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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