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Cardiovascular effects of exogenous adrenomedullin and CGRP in *Ramp* and *Calcrl* deficient mice

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ABSTRACT

Adrenomedullin (AM) and calcitonin gene-related peptide (CGRP) are potent vasodilator peptides and serve as ligands for the G-protein coupled receptor (GPCR) calcitonin receptor-like receptor (CLR/*Calcrl*). Three GPCR accessory proteins called receptor activity-modifying proteins (RAMPs) modify the ligand binding affinity of the receptor such that the CLR/RAMP1 heterodimer preferably binds CGRP, while CLR/RAMP2 and CLR/RAMP3 have a stronger affinity for AM. Here we determine the contribution of each of the three RAMPs to blood pressure control in response to exogenous AM and CGRP by measuring the blood pressure of mice with genetic reduction or deletion of the receptor components. Thus, the cardiovascular response of $Ramp1^{-/-}$, $Ramp2^{+/-}$, $Ramp3^{-/-}$, $Ramp1^{-/-}/Ramp3^{-/-}$ double-knockout (dKO), and $Calcrl^{+/-}$ mice to AM and CGRP were compared to wildtype mice. While under anesthesia, $Ramp1^{-/-}$ male mice had significantly higher basal blood pressure than wildtype males; a difference which was not present in female mice. Additionally, anesthetized $Ramp1^{-/-}$, $Ramp3^{-/-}$, and $Calcrl^{+/-}$ male mice exhibited significantly higher basal blood pressure than females of the same genotype. The hypotensive response to intravenously injected AM was greatly attenuated in $Ramp1^{-/-}$ mice, and to a lesser extent in $Ramp3^{-/-}$ and $Calcrl^{+/-}$ mice. However, $Ramp1^{-/-}/Ramp3^{-/-}$ dKO mice retained some hypotensive response to AM. These results suggest that the hypotensive effect of AM is primarily mediated through the CLR/RAMP1 heterodimer, but that AM signaling via CLR/RAMP2 and CLR/RAMP3 also contributes to some hypotensive action. On the other hand, CGRP's hypotensive activity seems to be predominantly through the CLR/RAMP1 heterodimer. With this knowledge, therapeutic AM or CGRP peptides could be designed to cause less hypotension while maintaining canonical receptor-RAMP mediated signaling.

1. Introduction

Adrenomedullin (AM) is a circulating multi-functional peptide and a known vasodilator [1]. AM is a member of the calcitonin peptide family along with amylin and calcitonin gene-related peptide (CGRP), a peptide considered to be one of the most potent endogenous vasodilators [2]. Circulating levels of AM are increased during many forms of cardiovascular disease [3] including heart failure where AM plays a cardioprotective role.

Exogenous administration of AM has beneficial hemodynamic/renal effects, improved cardiac output, and overall survival rates [4–8]. AM imparts these cardioprotective actions via numerous biological functions including vasodilation, diuresis, and natriuresis which are further augmented by inhibition of the renin-angiotensin-aldosterone system [4–7]. In addition, exogenous AM is known to play a protective role in lung injury and sepsis, perhaps through its anti-inflammatory and anti-microbial properties [9,10]. Given the beneficial properties of AM peptide, it may serve as a candidate therapeutic agent. However, the vasodilatory effects of AM may cause undesirable side-effects or be contraindicated in some patients. Therefore, a more thorough understanding of the mechanisms underlying the vasodilatory action of AM may allow for the development of effective therapeutics.

AM and CGRP are ligands for the G-protein-coupled receptor known as calcitonin receptor-like receptor (CLR = protein name; *Calcrl* = gene name). In the endoplasmic reticulum, CLR associates with one of three receptor activity modifying proteins (RAMPs) that confer ligand specificity and binding affinity [11]. On their own, CLR and RAMPs rarely migrate to cell surface, but as a complex

Abbreviations: AM, adrenomedullin; CGRP, calcitonin gene-related peptide; CLR, calcitonin receptor-like receptor protein; *Calcrl*, calcitonin receptor-like receptor gene; MAP, mean arterial pressure; RAMP, receptor activity modifying protein; dKO, double knockout.

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they translocate to the cell surface to interact with their respective ligands. Historically, the CLR/RAMP1 heterodimer is known as the CGRP receptor given its high ligand binding affinity for CGRP. CLR/RAMP2 and CLR/RAMP3 are known as the AM1 and AM2 receptors, respectively since AM is considered the primary ligand [12]. However, these receptor-RAMP heterodimers are not exclusively selective. AM can bind and activate the CGRP receptor and CGRP activates AM1 and AM2, though with lower potency than at the respective cognate receptors [13]

A 50% reduction in endogenous AM levels do not affect basal blood pressure in genetically heterozygous AM mice [14]. However, a bolus injection of AM causes dose-dependent hypotension [7,15]. The effects of exogenous AM on blood pressure have previously been examined using several genetic mouse models [16–18] and receptor inhibitors [19–21], but the relative contribution of each heterodimer to AM- and CGRP-induced hypotension has still not been fully explored or comparatively evaluated. Thus, the simultaneous examination of a comprehensive collection of genetic knockout mouse models for RAMPs and CLR could provide insight into the respective roles that CLR/RAMP1, CLR/RAMP2, and CLR/RAMP3 heterodimers play in the hypotensive responses to AM and CGRP.

In the current study, we examine the blood pressure and heart rate of mice under isoflurane anesthesia following intravenous injections of AM or CGRP. Gene disruption mouse models of *Calcrl*, *Ramp1*, *Ramp2*, and *Ramp3* were previously generated and are examined along with strain and age-matched wild type (WT) mice. Mice lacking *Calcrl* [22,23] and RAMP2 [18,23,24] are embryonic lethal, so these lines were examined as heterozygotes ($^{+/-}$).

2. Methods

2.1. Mice

All mice used in this study were between 8–16 weeks of age and are on the 129/S6-SvEv-TC1 background. The generation of *Ramp1*, *Ramp2*, *Ramp3* and *Calcrl* knockout mice were previously described [22,24,25]. All experiments were approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill.

2.2. Blood pressure measurements

Mice were anesthetized with 2% isoflurane gas. The left carotid artery was exposed and a suture was placed around the distal end of the artery. Then a loosely tied suture was placed around the proximal end. An occluding ligature was placed on the proximal end of the artery to minimize bleeding during catheter insertion. A small incision was made in the isolated carotid artery near the distal end and a SciSense mouse blood pressure transducer was inserted. The proximal occlusion was then removed as the transducer was advanced into the artery retrograde to the ascending aorta and tied in placed. Measurements were recorded through a Scisense ADVantage PV control unit onto Labscribe 2 software (iWorx Systems, Inc.).

2.3. Peptides

AM and CGRP peptides (Phoenix Pharmaceuticals) were dissolved in saline solution (0.9% Sodium Chloride, Hospira). A volume of 0.05 ml was used for all vehicle control and experimental dosage intravenous injections, and an additional 0.02 ml of saline was injected to clear the catheter of peptide. In experiments where two identical doses of AM were injected 20 min apart (Fig. 2), 12 nmol/kg of AM peptide was used per dose. In experiments where sequentially increasing doses of AM or CGRP were injected 5 min apart, 0.12 nmol/kg of peptide was used for the first dose, 1.2 nmol/kg for the second dose, 12 nmol/kg for the third dose, and 120 nmol/kg for the fourth and final dose.

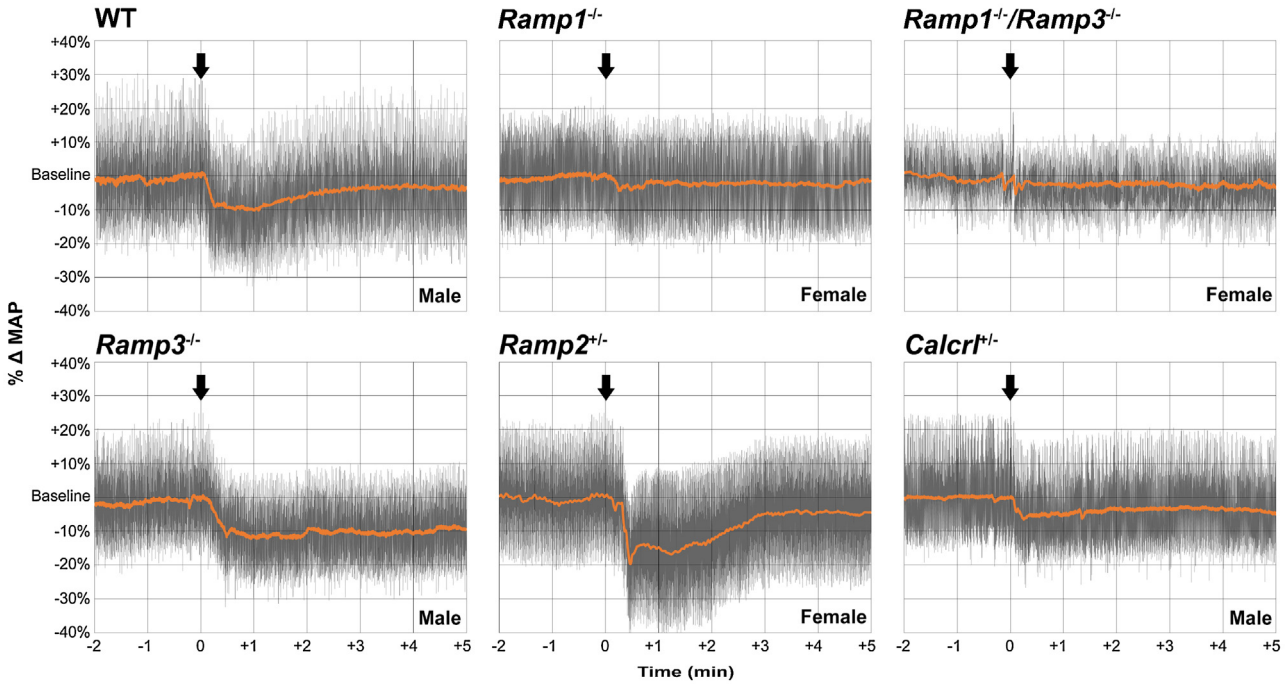

Fig. 1. Representative blood pressure charts from a single mouse receiving a 12 nmol/kg dose of AM converted to a percentage of baseline pressure (black), and the smooth line calculated from the chart (orange). Time 0 marks the time of AM injection. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)