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Original article

RGS5, RGS4, and RGS2 expression and aortic contractibility are dynamically co-regulated during aortic banding-induced hypertrophy

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Abstract

Overexpression of regulator of G protein signaling 5 (RGS5) in arteries over veins is the most striking difference observed using microarray analysis. The obvious question is what arterial function might require RGS5. Based on functions of homologous proteins in regulating cardiac mass and G-protein-coupled receptor (GPCR) signaling, we proposed that RGS5 and vascular expressed RGS2 and RGS4 could participate in regulating arterial hypertrophy. We used the suprarenal abdominal aorta banding model to induce hypertension and hypertrophy. All 3 RGS messages were expressed in unmanipulated aorta with RGS5 predominating. After 2 days, thoracic aorta lost expression of RGS5, 4, and 2. At 1 week, all three returned to normal, and at 28 days, they increased many fold above normal. Valsartan blockade of angiotensin II (angII)/angII type 1 receptor signaling prevented upregulation of RGS messages but only delayed mass increases, implying wall mass regulation involves both angII-dependent and angII-independent pathways. The abdominal aorta showed less dramatic expression changes in RGS5 and 4, but not 2. Again, those changes were delayed by valsartan treatment with no mass changes. Thoracic aorta contraction to GPCR agonists was examined in aortic explant rings to identify vessel wall physiological changes. In 2-day aorta, the response to Gαq/i agonists increased above normal, while 28-day aorta had attenuated induced contraction via Gαq/i agonist, implicating a connection between RGS message levels and changes in GPCR-induced contraction. *In vitro* overexpression studies showed RGS5 inhibits angII-induced signaling in smooth muscle cells. This study is the first experimental evidence that changes in RGS expression and function correlate with vascular remodeling.

Keywords: Regulator of G protein signaling; RGS5; Vascular hypertrophy; Hypertension; Aorta; Coarctation; GPCR; Artery contraction; Vascular remodeling

1. Introduction

Expression arrays identify the regulator of G protein signaling 5 (RGS5) as the most differentially expressed marker gene for arterial smooth muscle over vein [1,2]. The obvious functional question is, why should arteries as compared to veins differentially overexpress a regulator of G-protein-coupled signaling? Based on the known function of RGS proteins in regulating G-protein-coupled receptor (GPCR) signaling [3], the expression of RGS5 early in arterial development and function [4,5] and evidence that RGS proteins regulate remodeling in the heart [6,7], we postulated that RGS5 and its related RGS family members might play a role in the ability of the artery to contract and remodel [8]—functions that are critical to the ability of arteries to simultaneously maintain elevated pressure and flow.

The principal vasoconstrictors, serotonin, epinephrine, angiotensin (angII), vasopressin, endothelin-1 (ET-1), and thrombin, as well as the vasodilators, bradykinin and histamine, act via GPCRs regulated by different RGS proteins [3,9]. It was originally believed that termination of $G\alpha_{GTP}$ signaling generated by GPCR activation was dependent on self-catalyzed hydrolysis of $G\alpha_{GTP}$ to $G\alpha_{GDP}$, promoting dissociation of Gα subunits from their effectors and re-association with Gβγ and

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GPCR. However, the intrinsic Gα–GTPase activity is too low to account for the rapid physiological responses observed *in vivo* [10,11]. Instead, RGS proteins act as GTPase activating proteins (GAPs) for all Gα subfamilies. To date, more than 30 mammalian proteins with RGS sequences have been identified. RGS5 belongs to the nine-member R4-RGS subfamily together with RGS1, 2 , 3, 4, 8, 13, 16, and 18 [12]. Within this family, RGS5 appears to be the major RGS form present in the vasculature and typically marks smooth muscle cells (SMCs) and pericytes [1,2,4,13]. RGS5, like RGS4, RGS2 and other R4s, is a GAP for Gαq and Gαi, but not Gαs or Gα12/13 [4,14,15]. GAP activity for Gαq and Gαi implies that RGS5 may regulate GPCR-induced vessel wall contractile signaling. Recently, RGS5 was shown in human genome-wide linkage association studies to be located at a maximum logarithm of odds (MLOD) peak region for essential hypertension [16].

The present study is the first to show that RGS5, 4, and 2 expression levels change dramatically during arterial remodeling following suprarenal aortic banding and that these changes correlate with changes in contractile responses to vasoactive agonists. Additionally, we show that RGS5 inhibits angII-induced hypertrophic changes in vascular smooth muscle cells. These results support the hypothesis that R4-RGS proteins in the arterial wall may serve as molecular controls over vascular contractibility and remodeling.

2. Materials and methods

2.1. Aortic banding model

Aortic banding was performed on rats as previously described (Online Supplement (OLS) Section I, http://www.sciencedirect.com/science/journal/00222828) [17]. Briefly, male Sprague–Dawley (SD) rats (300–350 g, Charles River Laboratories, St. Constant, PQ, Canada) were anesthetized, the abdominal aorta was exposed through an incision 1.0 cm below the diaphragm. The aorta was sham-operated or banded using a blunted 20-gauge needle just above the renal arteries and fixed tightly to the aorta with silk thread; the 20-gauge needle was then removed, resulting in the constriction of the aorta. Valsartan, a selective angiotensin type 1 receptor (AT_1R) antagonist (30 mg/kg/day, p.o.), or placebo treatment was started on the day of the surgery and adjusted twice a week. Food and water were administered *ad libitum*. Buprenorphine (0.3 mg/kg s.c.) was used as a postoperative analgesic. Rats were anesthetized with a cocktail consisting of ketamine (50 mg/kg; MTC Pharmaceuticals, Cambridge, ON, Canada), xylazine (5 mg/kg; Bayer, Etobicoke, ON, Canada), and acepromazine (2.5 mg/kg; Ayerst Laboratories, Montreal, ON, Canada), then injected with 100 mM $CdCl_2$ via the vena cava to induce a diastolic arrest. Both thoracic and abdominal aortas were then rapidly isolated. After the remaining aortic tissue was stripped of adventitia and endothelium and snap-frozen in liquid nitrogen, RNA was isolated as previously described [1]. The procedures conformed to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and were approved by the local Institutional Animal Care Committee.

2.2. Quantitative real-time RT-PCR (Taqman)

Two-step real-time RT-PCR (see Table 1 for primers/probes) was used to quantitatively measure mRNA levels of RGS5, RGS4, and RGS2 in both thoracic and abdominal aorta (OLS Section II, http://www.sciencedirect.com/science/journal/00222828). All samples were amplified in duplicate and averaged; averages were then taken using 5 to 6 animals per condition. Data are presented graphically with standard error of the mean (SEM). The calculated mRNA levels were normalized by the amount of 18S rRNA, and the final data were expressed as a percentage of the control group.

2.3. In vitro measurement of aortic contraction

Aortic contractions in response to different agents used were measured in an aortic ring bath as described previously [18]. Isometric contractions were measured using isometric force transducers (Harvard Apparatus, Montreal, Canada) and a BIOPAC System data acquisition and analysis system (Harvard Apparatus, Montreal, Canada). To test the relaxation effect of isoproterenol and forskolin, cumulative concentrations of these agents were given to 100 nM phenylephrine (PE) pre-contracted rings. The vasoactive response of different agents was expressed as a percentage of maximal tension induced by 70 mM KCl (OLS Section III http://www.sciencedirect.com/science/journal/00222828). Data provided are the average of data from 3 to 5 animals, using SEM.

2.4. Retrovirus construction

Briefly, to make pBMN-hRGS5-I-EGFP and pBMN-I-rRGS5-EGFP expression retroviruses, the human RGS5 cDNA

Table 1
Primer pairs and probes used in Taqman

Target mRNA	Sequence of primer pairs and probes	cDNA template
RGS2		AF279918
Forward primer	5′-ATTGGAAGACCCGTTTGAGCTA-3′	
Reverse primer	5′-TTCCTCAGGAGAAGGCTTGATAA-3′	
Taqman probe	5′-6FAM-ACTCCTCTACTCCTGGGAAGCCCA-TAMRA-3′	
RGS4		AF117211
Forward primer	5′-TTCACACAGCAAGAAGGACAAAG-3′	
Reverse primer	5′-CGATTCAGCCCATTTCTTGAC-3′	
Taqman probe	5′-6FAM-TGGCTCACCCTCTGGCAAGTTACT-TAMARA-3′	
RGS5		AF241259
Forward primer	5′-TCCATATAATGAGAAGCCGGAGAA-3′	
Reverse primer	5′-TGAAGCTGGCAAATCCGTAGTT-3′	
Taqman probe	5-6FAM-TGCCAAGGCGCACAAGCCCT-TAMRA-3′	