Dissociation of Coronary Artery Contractile Hyperreactivity From Hypertension

Wen Su, Zhenheng Guo, Christian F. Deschepper, David C. Randall, and Ming C. Gong

Background: Both coronary artery contractile hyperreactivity and hypertension are associated with increased coronary artery disease. It is not known how coronary artery contractile hyperreactivity relates to hypertension. The current study tests the hypothesis that coronary artery contractile hyperreactivity can be dissociated from hypertension and therefore may contribute to the etiology of CAD independent of hypertension.

Methods: The contractile responses to 5-hydroxytryptamine (5-HT) and guanosine triphosphate (GTP) were determined in intact (nonpermeabilized) and α-toxin−permeabilized coronary artery strips and small mesenteric artery strips isolated from four rat strains: spontaneously hypertensive rats (SHR), Wistar-Kyoto rats (WKY), WKY-derived hypertensive rats (WKHT), and WKY-derived hyperactive rats (WKHA).

Results: The SHR and WKHT were hypertensive, whereas the WKY and WKHA subjects were normotensive. The coronary artery contractile reactivity to 5-HT was significantly higher in SHR when compared with WKY. The coronary artery contractile reactivity was of similar magnitude in WKHA and WKHT and was intermediate between that of SHR and WKY rats. GTP-induced Ca²⁺ sensitization of contractions were significantly greater in SHR than in WKHT, WKHA, and WKY; in comparison, no significant difference was found among WKHT, WKHA, and WKY. In contrast to the findings in coronary arteries, there was no significant difference in 5-HT−induced contractions in small mesenteric artery strips isolated from SHR and WKY.

Conclusions: Coronary artery contractile reactivity to 5-HT does not correlate entirely with blood pressure (BP) values. In addition, G-protein−mediated Ca²⁺ sensitization of contraction was increased and contributed to the coronary artery contractile hyperreactivity in SHR. Am J Hypertens 2003;16:570–576 © 2003 American Journal of Hypertension, Ltd.

Key Words: Coronary artery hyperreactivity, coronary heart disease, hypertension, Ca²⁺ sensitization of contraction.

Coronary artery hyperreactivity is a phenomenon in which localized regions in coronary arteries display enhanced contractions compared with adjacent regions to a variety of vasoconstrictor substances, including 5-hydroxytryptamine (5-HT). This phenomenon can elicit severe and sustained coronary artery contraction, which in turn plays an important role in the pathogenesis of coronary artery disease (CAD). There is ample evidence suggesting a direct and continuous association between prevailing BP and the incidence of CAD. However, in humans with high BP, a reduction of BP alone is not sufficient to reverse completely the hypertension-associated increased risk of CAD. The functional organization of the contributions of coronary artery hyperreactivity and systemic hypertension to the etiology of CAD is not known. One possibility is that the hypertensive state enhances coronary artery contractility, which in turn leads to an increased incidence of CAD. Conversely, a general increase in vascular smooth muscle reactivity, including that of the coronary vessels, could produce hypertension, which would then be reflected in CAD. Neither of these serial scenarios allows for the possibility of an independent contribution by hypertension or by coronary artery hyperreactivity to the etiology of CAD. Alternately, changes in the level of expression of a given gene, or set of linked genes, could cause both coronary smooth muscle hyperreactivity and hypertension, both of which could then independently potentiate the development of CAD.


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this were the case, both factors would contribute to the etiology of coronary disease but in a highly linked fashion. Finally, both factors could contribute by means of totally independent mechanisms to CAD.

The purpose of the present study was to test the hypothesis that the coronary artery contractile hyperreactivity and hypertension contribute to CAD through independent mechanisms by determining whether hypertension and coronary artery contractile hyperreactivity can be dissociated in four strains of rats.

To establish whether hypertension and coronary artery contractile hyperreactivity can be dissociated, we used four strains of rats: spontaneously hypertensive rats (SHR), Wistar-Kyoto rats (WKY), WKY-derived hypertensive rats (WKHT), and WKY-derived physically overactive rats (WKHA). The SHR is a widely used model of markedly elevated BP that resembles human essential hypertension in many ways. The WKY has been generally used as the normotensive control for SHR. Coronary arteries in SHR are reportedly hyperreactive to 5-HT–induced contractions when compared with WKY. Both SHR and WKY, however, are inbred strains generated by selective breeding approaches, so a multitude of genetic differences and therefore physiologic parameters (many more than the few that confer the BP phenotype), must thus be expected. It is consequently unclear whether the observed coronary artery contractile hyperreactivity to 5-HT in SHR as compared with WKY relates specifically to hypertension. The WKHT and WKHA strains were developed by crossing SHR with WKY, followed by recombinant selected inbreeding over 25 generations to separate hypertension and physical overactivity. Both of these two phenotypes are characteristic of SHR. Conversely, the WKHT strain is hypertensive but is not physically hyperactive, whereas the WKHA strain is physically hyperactive but normotensive. As such, the WKHA and WKHT strains constitute true mixes of the genomes of SHR and WKY. Used together, these four strains of rats represent one of the tools currently available to sort out which characteristics of SHR, previously elucidated using only WKY as controls, cosegregate with the hypertension.

Our results show that the coronary artery contractile hyperreactivity to 5-HT can be dissociated from hypertension. In addition, we show that G-protein–mediated Ca2+ sensitization of contraction is increased in SHR when compared with WKHT, WKHA, or WKY strains. These data indicate that treatment directed toward decreasing coronary artery contractile hyperreactivity, in addition to controlling BP, will aid in the treatment and prevention of CAD.

Material and Methods

Animals

Male SHR and WKY were obtained from Charles River (Wilmington, MA). Male WKHA and WKHT originated from colonies that are currently maintained at the Institut de Recherches Cliniques de Montréal (IRCM). These colonies are derived from breeding stock obtained from E.D. Hendley (Burlington, VT) and have been registered with the Institute of Laboratory Animal Resources of the National Research Council. All procedures were approved by the University of Kentucky Animal Care and Use Committee.

All rats were between the ages of 12 and 15 weeks at time of study. The animals were anesthetized with ketamine (75 mg/kg) and xylazine (6 mg/kg). A catheter was placed in the femoral artery and BP was recorded for a minimum of 0.5 h using a Cobe pressure transducer (Cobe Cardiovascular, Arvada, CO). The BP signal was digitized at 500 Hz and mean arterial pressure was computed beat-by-beat using a pentium computer and a data analysis program written in-house. Several days later the rats were re-anesthetized with halothane and killed by rapid exsanguination through the carotid artery. Heats and mesenteries were taken out immediately. The left anterior descending coronary artery (100 to 150 μm internal diameter) was dissected from the heart, and connective and cardiac muscle tissue were removed from the vessels under a stereomicroscope. Third order branches of mesenteric arteries (100 to 150 μm internal diameter) were dissected from the mesentery, and connective and fat tissue were removed from the vessels. The coronary and mesenteric arteries were cut into small spiral strips (2 to 3 mm long, 100 μm wide, and 20 to 30 μm thick). One to three strips from either coronary or mesenteric arteries were obtained from each animal. The endothelium was removed from the strips with razor blades. Successful denudation of endothelium was verified by the loss of acetylcholine-induced relaxation. Isometric tension was measured with a force transducer (Akers AE 801; AME, Horten, Norway) in a well on bubble plates as previously described.

Protocols

The muscle strips were mounted on a tension apparatus and stretched to approximately 1.5 times their resting length. They were then stimulated several times by incubation with a depolarization solution containing 143 mmol/L KCl until a stable contraction was obtained. Finally, a 5-HT dose-response curve was obtained by adding increasing cumulative concentrations of 5-HT. The high K+ depolarization solution–induced contractions were not different among SHR, WKY, WKHA, and WKHT (see Results). Therefore, the amplitude of 5-HT–induced contractions was normalized to the respective high K+–induced contractions in subsequent experiments to exclude the force variation caused by alteration of smooth muscle cell mass in different arterial strips. The amplitude of contractions was expressed as mean ± SEM.

Coronary artery strips were permeabilized by incubation with 17.5 μg/mL Staphylococcus aureus α toxin for 45 min (List Biological Laboratories, Campbell, CA) to
Table 1. Mean arterial blood pressures (mm Hg) in the four rat groups

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<th>WKY</th>
<th>WKHA</th>
<th>SHR</th>
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<td>95 ± 4 (10)</td>
<td>96 ± 2 (5)</td>
<td>117 ± 2*† (8)</td>
<td>115 ± 3*† (5)</td>
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WKY = Wistar-Kyoto rats; WKHA = WKY-derived hyperactive rats; SHR = spontaneously hypertensive rats; WKHT = WKY-derived hypertensive rats.

* P < .05 vs WKY; † P < .05 vs WKHA.

determine the G-protein-mediated sensitization of contraction to Ca2+. The intracellular Ca2+ stores were depleted by incubation with a Ca2+ ionophore (10 μmol/L A23187 for 10 min). The strips were then partially contracted by exposure to a submaximal concentration of Ca2+ (pCa6.5). Tautomycin or various concentrations of GTP were then added cumulatively under constant free Ca2+ (pCa6.5). The GTP- and tautomycin-induced contractions were normalized to the level of contraction obtained after a maximal concentration of Ca2+ to eliminate the variation due to the alteration of smooth muscle cell mass in different arterial strips.

Solutions

The normal external solution was a HEPES-buffered modified Krebs solution containing (mmol/L): Na+, 137.4; K+, 5.9; Ca2+, 1.2; Mg2+, 1.2; Cl−, 148.1; glucose, 11.5; and HEPES, 11.6 (pH 7.3 with NaOH at 20°C). The high potassium depolarizing solution (143 mmol/L) was made by replacing Na+ with an equivalent amount of K+. The normal relaxing solution was (in mmol/L): potassium methanesulfonate, 74.1; magnesium methanesulfonate, 2; MgATP, 4.5; EGTA, 2; creatine phosphate, 10; PIPES, 30 (pH 7.1 with KOH at 20°C). In the activating solution, 10 mmol/L EGTA was used, and a specified amount of calcium methanesulfonate was added to obtain a desired concentration of free Ca2+ ions. Ionic strength was kept constant at 0.2 mol/L by adjusting the concentration of potassium methanesulfonate.

Statistical Analyses

One-way analysis of variance was used to determine statistical significance of differences among the four groups of rats. This was followed by a post hoc Newman-Keuls test for comparison among multiple groups, when applicable.

Results

Arterial BP in SHR, WKY, WKHA, and WKHT Rats

Mean arterial BP measured before assessment of coronary artery and mesenteric artery contractile reactivity are summarized in Table 1. It is particularly noteworthy that the BP values for the SHR and WKHT were significantly greater than those for WKY and WKHA. In the present study, BP was measured directly using a femoral artery catheter and under anesthesia with ketamine and xylazine. The relatively lower BP values in the current study as compared with those generally reported in the respective studies in unanesthetized rats were probably due to ketamine and xylazine, which have been reported to decrease BP by 26% to 30%.

Dissociation Between Coronary Artery Contractile Hyperreactivity and Hypertension

We determined the dose-response curves of 5-HT contractions in coronary arteries from hypertensive SHR and WKHT, as well as from normotensive WKY and WKHA. As shown in the representative tension recordings (Fig. 1A), high K+ (143 mmol/L) depolarization-induced contractions that were of similar magnitude and not statistically significantly different among the four rat strains. In contrast, 5-HT induced contractions varied significantly among the four rat strains. The 5-HT–induced contractions were significantly larger in SHR than in WKY for all concentrations tested (Fig. 1A and 1B). The contractile reactivity to 5-HT in WKHA and WKHT was the same and intermediate between that of SHR and WKY; it was significantly lower than the response in SHR and significantly higher than the response in WKY. There was no significant difference in pD2 values (−logEC50) among the four rat strains. Importantly, there was no correlation between BP values and the contractile reactivity to 5-HT among different rat strains (correlation coefficient [r2] = 0.52, P = .27) (Fig. 1C).

To determine further whether the observed increase in contractile responses in coronary arteries was selective for 5-HT, we measured and compared contractions induced by tautomycin, a phosphatase inhibitor that induces contractions by directly inhibiting myosin phosphatase. There was no significant difference between SHR and WKY in coronary artery contractions induced by tautomycin (data not shown).

Increase of G-Protein–Mediated Ca2+ Sensitization of Contraction in Coronary Arteries

Increased cytoplasmic free Ca2+ concentration or increased sensitivity of the contractile apparatus to Ca2+ (also called Ca2+ sensitization) may have caused the observed increased coronary artery contractile responses to 5-HT. To test the potential role of increased G-protein activation–induced Ca2+ sensitization, we studied the dose-response curves of GTP-induced Ca2+ sensitization of contractions in α-toxin permeabilized preparations in the four strains of rats. α-Toxin creates small pores in the plasma membrane and allows one to study Ca2+ sensitization of contraction selectively by clamping the cytoplasmic free Ca2+ concentration. The addition of GTP in the
presence of constant free \(\text{Ca}^{2+}\) caused a dose-dependent contraction by sensitizing the contractile apparatus to \(\text{Ca}^{2+}\) in vessels from all four strains. Moreover, the GTP-induced \(\text{Ca}^{2+}\) sensitization of coronary artery contractions was significantly greater in SHR than in WKHT, WKHA, and WKY (Fig. 2). In contrast, GTP-induced \(\text{Ca}^{2+}\) sensitization in WKHA and WKHT was not different from that found in WKY (Figs. 1 and 2).

To determine whether the \(\text{Ca}^{2+}\) sensitivity of the contractile apparatus was increased in SHR and could thereby contribute to the increased GTP-induced \(\text{Ca}^{2+}\) sensitization in SHR, we compared the effect of a submaximal concentration of \(\text{Ca}^{2+}\) (pCa6.5) on the contractions of coronary arteries from SHR and WKY. At that \(\text{Ca}^{2+}\) concentration, coronary artery contraction was 17% ± 5.0% and 19% ± 4.4% (\(n = 4\) each, \(P > .05\)) of the maximal \(\text{Ca}^{2+}\)-induced contraction in SHR and WKY, respectively. These values were not significantly different.

We found that 5-HT–induced contractions were selec-
tively increased in coronary arteries. To determine whether 5-HT hyperreactivity is restricted to the coronary artery or whether it also exists in small arteries of a similar diameter from other vascular beds, we investigated 5-HT reactivity in small mesenteric arteries. Similar to what was observed in coronary artery, there is no significant difference in high \( \text{K}^+ \)-depolarization–induced contraction between the mesenteric arteries obtained from SHR and WKY (17 ± 4 mg vs 15 ± 2 mg). As shown in the right panel of Fig. 3, however, the amplitudes of 5-HT–induced contractions were not statistically significantly different in mesenteric arteries obtained from SHR and WKY. The overall response to 5-HT was much higher in the mesenteric arteries than in the coronary arteries (CA) (Fig. 3, left panel) when compared with their respective high potassium depolarization–induced contractions. We consistently failed to detect any difference in GTP-induced \( \text{Ca}^{2+} \) sensitization of mesenteric artery contractions between SHR and WKY (data not shown).

**Discussion**

A major finding of the current study is that coronary arteries obtained from SHR show contractile hyperreactivity to 5-HT when compared with similar arteries from WKY, but that 5-HT coronary artery reactivity was not proportional to BP values in SHR, WKY, WKHA, and WKHT. Indeed, the coronary artery reactivity to 5-HT was significantly higher in WKHA than in WKY, although BP values were almost identical in these two strains. Likewise, the coronary artery reactivity to 5-HT was significantly lower in WKHT than in SHR, despite almost identical BP values. Finally, the coronary artery reactivity to 5-HT was almost identical between the WKHT and WKHA, despite the fact that BP values were significantly higher in WKHT than in WKHA. Thus, these findings clearly show that BP values do not directly correlate with the coronary arterial contractile reactivity to 5-HT.

The contractile response to 5-HT was specifically of interest to us because several lines of evidence suggest that 5-HT, which is locally released from aggregated platelets, is a candidate intrinsic stimulator of coronary vasoconstriction. For instance, 5-HT concentrations are elevated in the coronary sinus in patients with coronary artery disease. Likewise, the action of ergonovine is believed to be mediated mainly through the activation of 5-HT receptors located on smooth muscle. Ergonovine is a powerful and widely used agent for the provocation of coronary spasms in susceptible patients with variant angina, and ergonovine-induced attacks are remarkably similar to spontaneous episodes. Finally, in patients with angina,
5-HT infusion produces either intense coronary artery vasoconstriction or, in some cases, occlusive coronary artery spasms.15

The molecular mechanisms underlying 5-HT hyperreactivity in coronary arteries remain to be established. The hyperreactivity may be explained by changes at the level of 5-HT receptors or in the signal transduction pathway downstream from the 5-HT receptors. Substantial in vitro evidence indicates that under certain experimental circumstances (namely, precontraction with a non–5-HT receptor agonist), inactive or silent 5-HT receptor subtypes become active, and a contractile response to 5-HT is either unmasked or substantially enhanced.16 In SHR, WKHT and WKHA, the availability of some agonists, such as endothelin or angiotensin II, may be increased and may thus activate 5-HT receptors by such a mechanism. In addition, the signal transduction pathway downstream from the 5-HT receptor must also be involved in SHR, as direct G-protein activation induces significantly greater contractions in coronary arteries from SHR than in their WKY counterparts (Fig. 2). The contractile abnormality, whatever its nature, probably occurs upstream of the myosin light chain kinase and myosin phosphatase, inasmuch as the contractions induced by direct activation of the myosin light chain kinase with Ca2+ (pCa6.5), or the direct inhibition of myosin phosphatase with tautomycin, are similar between SHR and WKY.

Contractile agonists induce smooth muscle contractions by increasing cytoplasmic free Ca2+ levels and by increasing the sensitivity of the contractile apparatus to Ca2+ (Ca2++ sensitization).17, 18 That GTP-induced greater Ca2+ sensitization mediated contractions in SHR suggests that the Ca2+ sensitization pathway is up-regulated in SHR. Interestingly, in the coronary arteries obtained from WKHT and WKHA, the reactivity to 5-HT is higher than those obtained from WKY (Fig. 1). However, the GTP-induced Ca2+ sensitization of contraction is similar among WKHT, WKHA, and WKY strains (Fig. 2). This suggests two possibilities. The first is that the G-protein–mediated Ca2+ sensitization did not contribute to greater 5-HT–induced coronary artery contractions found in WKHT and WKHA when compared with WKY. The second possibility is that 5-HT–induced Ca2+ sensitization is actually higher in SHR than in WKY and contributes to the observed increased 5-HT contractile response in intact tissue. However, GTP activates all G-proteins including stimulatory and inhibitory G-proteins. The functions of these G-proteins may be differentially altered in WKHT, WKHA, and WKY, which therefore could conceal the change in the G-protein that is coupled to the 5-HT receptor. Determination of the 5-HT–induced Ca2+ sensitization of contraction in different strains of rats is required to distinguish these two possibilities. Furthermore, the reactivity to 5-HT in WKHA and WKHT was intermediary between that of the SHR and the WKY strain. This, as well as the observation that the G-protein–mediated Ca2+ sensitization was up-regulated in SHR but not in WKHT and WKHA, suggest that reactivity to 5-HT is a phenotype that is determined by multiple genes and multiple mechanisms.

The lack of correlation between the BP values and the amplitude of coronary artery contractile reactivity suggests that hypertension and coronary artery hyperreactivity can contribute independently to the etiology of CAD. Therefore, to prevent and to treat CAD more effectively, decreasing the coronary artery contractility should be considered in addition to controlling BP. In particular, in view of the increased GTP-induced Ca2+ sensitization of contraction in coronary artery from SHR, use of therapy directed at inhibiting G-protein–mediated Ca2+ sensitization, in addition to the standard calcium antagonist, may aid in decreasing the coronary artery contractile hyperreactivity. The SHR are hypertensive and their coronary arteries are hyperreactive to 5-HT–induced contraction. Therefore, when used with WKHT, the WKHA, WKY, and SHR serve as a very useful means for sorting out whether coronary artery hyperreactivity links with hypertension. However, we recognize that, in addition to hypertension, many factors that contribute to the cause of human CAD that may not be present in SHR. Furthermore, there may be unknown differences in coronary artery characteristics between rat and humans. Therefore, more human studies are required before one can extrapolation the current results to treatment of human CAD.

In summary, our results suggest that coronary arteries are selectively hyperreactive to 5-HT in SHR, and that this hyperreactivity does not correlate with BP levels in SHR, WKY, WKHT, and WKHA. Multiple mechanisms including G-protein–mediated Ca2+ sensitization probably underlie the observed 5-HT hyperreactivity, at least in the SHR strain. Our data implicate decreasing the coronary artery contractility, in addition to control of hypertension, in the prevention and treatment of CAD.

References


