Enhanced Vascular Reactivity to Mastoparan, a G Protein Activator, in Genetically Hypertensive Rats

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Abstract Vascular smooth muscle from stroke-prone spontaneously hypertensive rats has an increased responsiveness to the vasoconstrictors angiotensin II and serotonin. This abnormality is postulated to contribute to the hypertension characteristic of this strain of rats. We hypothesized that a portion of the increased responsiveness may be due to altered function of G proteins. This hypothesis was tested using mastoparan, a peptide that mimics ligand-bound receptors to stimulate G proteins directly. In addition, we investigated the mechanism of mastoparan-induced contraction of vascular smooth muscle. Changes in isometric tension were recorded in denuded carotid artery strips from hypertensive and normotensive (Wistar-Kyoto) rats. Vascular strips from the hypertensive rats had a significantly greater response to mastoparan at all concentrations between $10^{-8}$ and $10^{-5}$ mol/L. A G protein inhibitor, N-ethylmaleimide ($10^{-3}$ mol/L), attenuated the response to mastoparan ($10^{-7}$ mol/L) (67±4% of control response), whereas pertussis toxin treatment did not. Inhibition of phospholipase C also significantly decreased the mastoparan-induced response (23±12% of control), and nifedipine ($10^{-3}$ mol/L), a calcium channel blocker, completely blocked the mastoparan-induced contraction. Indomethacin treatment did not affect the mastoparan contraction even though mastoparan has been shown to stimulate phospholipase A$_2$ in other cell types. In conclusion, we observed an increased response in carotid arteries from genetically hypertensive rats to a pharmacological intervention that appears to act via G protein-linked phospholipase C stimulation and L-type calcium channel activation, suggesting that the increased vascular reactivity in stroke-prone spontaneously hypertensive rats is due in part to altered function of G proteins.

Key Words • rats, inbred SHR • phospholipase C • muscle, smooth, vascular • calcium channels

Hypertension is associated with altered vascular reactivity to many agonists. The inbred strain of stroke-prone spontaneously hypertensive rats (SHRSP) specifically exhibits increased sensitivity to vasoconstrictors and a decreased response to vasodilators.1 Presently, the mechanisms responsible for this augmented responsiveness are not clear. Potential sites of modification in vascular tissue that could lead to increased responsiveness include increased number or affinity of agonist receptors, altered function or number of intramembrane signal transduction molecules (ie, G proteins), elevated function of intracellular second-messenger-generating enzymes, and enhanced sensitivity of contractile proteins to second-messenger signals.

Previous studies have shown that although receptor upregulation occurs in some models of hypertension, it is not sufficient to explain the differences in contractility.2 Furthermore, the sensitivity of contractile proteins to calcium is not elevated in genetic hypertension in the rat,3 nor is there a difference in the function of second-messenger-generating enzymes4,5 or in the amount of G proteins present in vascular smooth muscle of hypertensive rats.6,7 However, the function of G proteins in vascular tissue has not been compared between hypertensive and normotensive animals.

The purpose of this study was to examine the function of G proteins in isolated vascular tissue from hypertensive and normotensive rats and to test the hypothesis that altered function of one or more G proteins contributes to the increased vascular reactivity in SHRSP. The G protein stimulator mastoparan was used to contract denuded carotid artery strips isolated from hypertensive and normotensive rats. Mastoparan is a peptide originally isolated from wasp venom and, unlike A1F4,8 which nonselectively stimulates G proteins in vascular preparations,8 mastoparan has some selectivity for G$_2$ and G$_i$ compared with G$_s$ (the affinity for G$_s$ has not previously been investigated).9 Mastoparan should therefore prove to be a novel and useful probe to investigate G protein–mediated contractions in vascular smooth muscle.

Methods

Reagents

Mastoparan was purchased from Peptides International; phenylephrine (Elkins-Sinn Inc) and acetylcholine (Sigma) were purchased from University of Michigan Hospital Stores, Ann Arbor, and all others from Sigma.

Animals

SHRSP and normotensive Wistar-Kyoto (WKY) rats were obtained from a colony in the Department of Anatomy and Cell Biology at the University of Michigan. Some WKY rats used in the concentration response experiments were from Harlan Sprague Dawley, Inc. Systolic blood pressure was measured using the tail-cuff method with a pneumatic pulse transducer (Narco Biosystems). All protocols used in these studies were reviewed by the University of Michigan Animal Use Committee and were in compliance with institutional guidelines.
Preparation of Tissues

Rats were anesthetized with sodium pentobarbital (50 mg/kg IP), and carotid arteries were dissected and placed in physiological saline solution (PSS) containing (mmol/L) NaCl 130, KCl 14.7, KH₂PO₄ 1.18, MgSO₄ 7H₂O 1.17, NaHCO₃ 14.9, dextrose 5.5, CaNa₂ EDTA 0.026, and CaCl₂ 1.6. Vessels were cleaned, cut into helical strips (0.8 x 10 mm), and mounted in silicon-coated tissue baths filled with PSS maintained at 37°C and aerated with 95% O₂-5% CO₂. Endothelium was removed from strips by threading vessels on a metal stylet. SHRSP and WKY strips were paired in baths and force recorded with an FT03D Grass Instruments transducer. Strips were stretched to an optimal passive force for maximal active force development (500 mg) and equilibrated 2 hours before tissues were contracted with phenylephrine (10⁻³ mol/L) and the presence of endothelium tested by relaxation to acetylcholine (10⁻⁴ mol/L). Strips that relaxed more than 30% of the phenylephrine contraction were not used.

Contractile Experiments

Time Course of Contraction

Mastoparan (10⁻³ mol/L) or vehicle (water) was added to the tissue bath and force recorded for 1 hour.

Concentration-Response Curves

Cumulative concentration-response curves were generated for mastoparan (10⁻⁸ to 10⁻² mol/L) with peptide added at 20-minute intervals. Maximal contractions to serotonin (10⁻⁴ mol/L) or phenylephrine (10⁻⁵ mol/L) were evaluated before mastoparan contraction. Additional concentration-response curves were generated in the presence of the phospholipase C (PLC) antagonist 2-nitro-4-carboxyphenyl N,N-diphenylcarbamate (NCDC, 10⁻⁵ mol/L) or vehicle (ethanol).

Effect of N-Ethylmaleimide and Indomethacin

Tissues from the same animals were hung in two separate baths and contracted with mastoparan (10⁻³ mol/L) for 1 hour before indomethacin (10⁻³ mol/L) or N-ethylmaleimide (NEM, 10⁻⁴ mol/L) was added to one bath and vehicle (ethanol or distilled water) added to the other bath. Force was recorded for an additional 30 minutes.

Effect of Pertussis Toxin

Tissues were dissected as described above, and one carotid artery from each animal was incubated in normal PSS and one in PSS containing 100 ng/mL pertussis toxin for 24 hours at 4°C. Strips then were cut and mounted, and contractile responses to serotonin (10⁻² mol/L), phenylephrine (10⁻⁴ mol/L), and mastoparan (10⁻³ mol/L) were recorded.

Nifedipine Blockade

Vascular strips from the same animal were incubated for 30 minutes with nifedipine (10⁻⁵ mol/L) or vehicle (ethanol) before the addition of mastoparan (10⁻⁵ mol/L). Force was measured after 20 minutes.

Statistics

Concentration-force curves were generated for each individual strip and the EC₅₀ calculated graphically. Student's t tests were used to compare means. A Bonferroni correction was used when more than three comparisons were made.

Results

Systolic blood pressure and body weight were significantly different between hypertensive and normotensive strains (SHRSP, 183±3 mm Hg, 275±3 g; WKY, 111±3 mm Hg, 303±5 g) in animals matched for age (15 to 17 weeks). In both WKY and SHRSP carotid arteries, mastoparan (10⁻⁵ mol/L) caused a slowly developing contraction (Fig 1). At this concentration, strips from SHRSP developed significantly more force than those from WKY rats, although the time course and rate of force development were not different between strains. Maximal contraction was achieved by 20 minutes and was sustained for the remainder of the 60-minute recording period. In the concentration-response study, the response to mastoparan was significantly different between SHRSP and WKY at all concentrations between 10⁻⁸ and 10⁻³ mol/L (Fig 2).

NEM causes hydrolysis of disulfide bonds and eliminates some G protein-mediated responses. In SHRSP carotid arteries contracted with mastoparan (10⁻⁵ mol/L), 30 minutes of exposure to NEM (10⁻⁵ mol/L) caused a small but significant decrease in force (Fig 3), suggesting that mastoparan contraction is partially mediated by NEM-sensitive G proteins. Interestingly, treatment of arteries with pertussis toxin did not affect mastoparan contraction (Fig 3), nor did it affect responses to serotonin or epinephrine (data not shown). The effect of the antagonists was not examined in WKY tissues because most of the tissues did not respond to this concentration of mastoparan.

Fig. 1. Plot shows time course of mastoparan (10⁻⁵ mol/L) contraction. Each point represents the mean of five (Wistar-Kyoto, WKY) or six (stroke-prone spontaneously hypertensive, SHRSP) rats; error bars represent SEM. Bar graph on right depicts maximal response to 90 mmol/L KCl in the same tissues. Time to maximal force development was not different between groups of tissues. Asterisks designate points where force was significantly greater in SHRSP compared with WKY rats (P < 0.05).

Fig. 2. Plot of cumulative concentration response curves to mastoparan. Points represent the mean of seven rats; error bars depict SEM. Inset bar graph represents maximal response to phenylephrine (PE, 10⁻³ mol/L) in tissues used. Asterisks indicate significant difference between groups (P < 0.05). SHRSP indicates stroke-prone spontaneously hypertensive rats; WKY, Wistar-Kyoto rats.
Treatment of vascular strips from SHRSP with nifedipine completely blocked the response to mastoparan (10^{-7} \text{ mol/L}), implicating the involvement of L-type calcium channels in mastoparan contractions (Fig 3). The lack of complete reversal by nifedipine at higher concentrations of mastoparan (10^{-5} \text{ mol/L}, 63±3 mg treated versus 180±3 mg untreated) suggests that an additional mechanism is activated at this higher concentration. In contrast, indomethacin (10^{-5} \text{ mol/L}) did not reverse the mastoparan-induced contraction (10^{-7} \text{ mol/L}) (Fig 3) even though mastoparan has been shown to stimulate phospholipase A\textsubscript{2}. PLC inhibition with NCDC shifted the concentration response curve to the right in SHRSP and eliminated the response in WKY tissues (Fig 4). This supports a role for PLC activation in mastoparan-induced contraction of isolated carotid arteries.

**Discussion**

G proteins in vascular smooth muscle are activated by \(\alpha\)-adrenergic agonists, angiotensin II, arginine vasopressin, serotonin, and endothelin. These agonists, with the exception of \(\alpha\)-agonists, all have been shown to stimulate G protein activation of PLC and to initiate the generation of inositol phosphates (ie, inositol 1,4,5-trisphosphate [IP\textsubscript{3}] and diacylglycerol (reviewed in References 13 and 14).

In hypertension, vascular smooth muscle has been shown to have increased sensitivity to these \(G_q\)-linked vasoconstrictors and elevated levels of phosphoinositide hydrolysis after agonist stimulation.\textsuperscript{15,16} However, immunohistochemical measurement of G proteins in vascular tissue\textsuperscript{1,2} failed to show differences in the quantity of \(\alpha\)-subunits between hypertensive and normotensive rats. This is in contrast to reports of elevated levels of mRNA for G proteins in spontaneously hypertensive rats\textsuperscript{17} and to the well-characterized enhanced sensitivity of vascular tissue to G protein-linked agonists in SHRSP. Previous reports, therefore, have not been able to clarify the link between elevated vascular reactivity in hypertension and changes in the vascular content of G proteins.\textsuperscript{14} Notably, no studies address possible differences in the inherent reactivity of G proteins in hypertensive and normotensive rats.

The present studies used a functional rather than biochemical measure of G protein activity to provide direct evidence of increased G protein responsiveness in vascular tissue from hypertensive animals. The results support the hypothesis that the increased contractility characteristic of blood vessels chronically exposed to elevated blood pressure is produced by an enhanced response to G protein stimulation. Moreover, our findings demonstrate for the first time that mastoparan is useful for studying G protein contraction of vascular myocytes.

Previous studies in isolated membranes have established the mechanism for interaction of mastoparan with G proteins. The peptide has been shown to intercalate into membranes in an \(\alpha\)-helical formation, binding to G proteins at the same site as ligand-bound receptors and mimicking receptor-mediated activation.\textsuperscript{18} The ability of mastoparan to penetrate membranes and activate G proteins in intact cells has been demonstrated in mast cells,\textsuperscript{12} pulmonary artery endo-
thelial cells, and human astrocytoma cells. In phospholipid bilayers, mastoparan exhibits a greater affinity for Gq and Gs than for Gi, although the affinity for Ga proteins has not been examined. In the current experiment, pertussis toxin treatment at a concentration that ADP ribosylates endogenous G proteins in intact vascular preparations did not affect the contractile response to mastoparan, so the G protein activated in vascular smooth muscle cells does not appear to be Gi or Gs. This is further supported by the observation that NEM, which has been shown to inactivate primarily Gi, only partially reversed the contraction. Since only members of the Ga and Gs families have been linked to PLC activation (reviewed in Reference 13) and the PLC inhibitor NCDC inhibits mastoparan contraction at a concentration shown to inhibit phosphoinositide production, the mastoparan-induced contraction of vascular tissue appears to be mediated by a member of the Gs family.

Inhibition by nifedipine also is consistent with mastoparan acting via Gi, stimulation of IP3 generation, since IP3-initiated contractions have been shown to be maintained through Ca2+ influx through one or more plasma membrane Ca2+ channels (reviewed in Reference 22). Moreover, mastoparan has been shown to stimulate G protein–dependent IP3 production in other cells and to cause a concentration-dependent increase in intracellular Ca2+. Therefore, the enhanced mastoparan response in SHRSP combined with previous observations of elevated inositol generation and calcium signaling in SHRSP vascular myocytes support the hypothesis that the responsiveness of Ga is elevated in genetic hypertension.

In addition to its effects on G proteins, mastoparan has been shown to stimulate several other cellular processes that also mediate vascular smooth muscle contraction. For example, mastoparan has been shown to activate phospholipase A2 (5×10−9 mol/L). This effect did not contribute to contraction in the current studies, however, since indomethacin did not alter the response to mastoparan. In addition, mastoparan has been shown to modulate Ca2+-sensitive K+ channels (0.5 to 1×10−9 mol/L) and increase L-channel–independent Ca2+ permeability of cell membranes (>10−5 mol/L), effects that may have contributed to the mastoparan contraction at high concentrations (10−5 mol/L), since nifedipine did not totally block the contraction at this concentration. However, since nifedipine totally blocked the contraction to lower concentrations of mastoparan, our observations do not support a contribution by these mechanisms at lower mastoparan concentrations. Finally, mastoparan has been shown to inhibit protein kinase C activation (2 to 3×10−6 mol/L) and to directly stimulate PLC (5×10−6 mol/L). The inhibition of protein kinase C diminishes vascular smooth muscle contraction so that this action, if it was present, masked rather than contributed to the mastoparan contraction. The stimulation of PLC directly is consistent with our results and may have contributed to the non-NEM–sensitive portion of the contraction. Since these non–G protein–mediated effects of mastoparan occur primarily at high concentrations and only the contractile responses observed between 10−5 and 10−6 mol/L were used to evaluate the mechanism of mastoparan contraction, the contraction by mastoparan appears to be mediated primarily by direct stimulation of G proteins.

In summary, significant differences were observed in the contraction of arteries from SHRSP and WKY rats to mastoparan. These contractions were inhibited by NEM, NCDC, and nifedipine. The current studies in combination with previous observations of elevated inositol phosphate production support the hypothesis that increased responsiveness of G proteins leads to elevated PLC activity and may contribute to the elevated vascular responsiveness of SHRSP. Endogenous regulators of G protein function have not been well characterized, although it is known that acylation, phosphorylation, prenylation, and ADP ribosylation modify G protein function. Therefore, the abnormalities in vascular reactivity documented in SHRSP may be due to a change in the level of regulatory modifications of the G protein(s) mediating smooth muscle contraction, resulting in an increased responsiveness of the signal transduction system to hormone stimulation.

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References


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