Disruption of microtubular network attenuates histamine-induced dilation in rat mesenteric vessels

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Submitted 8 March 2004; accepted in final form 6 October 2004

EUKARYOTIC CELLS CONTAIN a complex set of protein fibers found in the cytoplasm—the cytoskeleton. The cytoskeleton contains three major classes of fibers: actin microfilaments, microtubules (MTs), and intermediate filaments. MTs, a major component of the cytoskeleton, are cylindrical structures composed of two types of protein, α- and β-tubulin. In general, the biological functions of MTs are partially based on the ability of tubulin to polymerize and depolymerize. In living cells the MTs are in a dynamic process between tubulin dimers and tubulin polymerized into MTs (8).

MTs play a role in a variety of cellular functions, including mitosis, trafficking of proteins, shape, and signaling processes (16). In recent years, the role of the MT network in transduction processes in the cell has been the object of several studies. It has been shown that MTs are associated with a variety of proteins involved in cell signaling, including adenylate cyclase (42), phospholipase C (41), and small G proteins (6).

The use of various pharmacological agents that affect the polymerization state of the MT network has yielded many novel relationships between MTs and those cell signaling pathways. Nocodazole and colchicine, two destabilizing agents, bind to the colchicine binding site of tubulin, shifting the dynamic state of MTs toward depolymerization by preventing assembly of tubulin subunits (4, 18).

Recently, it was shown that MTs participate in the control of vascular reactivity. Several studies demonstrated that disruption of MTs with nocodazole increases the contractile response in a variety of vessels and cells (23, 38–40, 47). Data from our laboratory (26) also suggested that disruption of the MT network increases the vasoconstrictor response to phenylephrine in the mesenteric arterial bed from normotensive rats, and this effect seems to be associated with the RhoA/Rho kinase pathway (6, 40).

The modulatory role of the MT network on the vasodilation response has also been evaluated. The results demonstrated that MT disruption decreases flow-dependent dilation of arterioles, does not affect the vasodilator response induced by agonists such as acetylcholine (ACh) and sodium nitroprusside (SNP) (19, 50), but does attenuate adenosine-induced arteriolar vasodilation (40).

We hypothesized that MT disruption affects endothelium-dependent nitric oxide (NO)-mediated smooth muscle relaxation. Therefore, the aim of this study was to investigate the effect of MT-destabilizing agents on vasodilator responses induced by histamine, ACh, SNP, and pinacidil in the isolated mesenteric arterial bed of normotensive rats.

METHODS

Isolated mesenteric arterial bed. Animal procedures were performed in accordance with the “Guiding Principles for Research Involving Animals and Human Beings” of the American Physiological Society. Male Wistar rats weighing 230–250 g were anesthetized with ether, and the mesenteric arterial bed was isolated as previously described (25, 32). The vessels were perfused at a constant flow of 2.5 ml/min with a physiological salt solution (PSS; in mM: 118.0 NaCl, 3.0 CaCl2, 1.43 MgCl2, 25.0 NaHCO3, 1.17 KH2PO4, 11.0 glucose, and 0.03 Na2EDTA) kept at 37°C and pH 7.4 and aerated with 95% O2-5% CO2. Changes in perfusion pressure were continuously monitored through a data-acquisition system (DATAQ). Dose-response curves to vasodilator agents were performed after a 30-min stabilization period in half-maximally preconstricted preparations with either phenylephrine (15–30 μM) or high-K+ (50 mM) modified PSS.
solution. Some preparations were perfused with 1 ml of deoxycholic acid (1.0 mg/ml), and the complete absence of functional endothelium was confirmed by injection of 1.2 nmol of ACh. Preconstriction (perfusion pressure level ~100 mmHg) was achieved by continuous perfusion of a modified high-K⁺ (50 mM) PSS solution or PSS containing variable concentrations of phenylephrine (15–30 μM). The dose-response curves to the various vasodilator agents were performed after bolus injection into the perfusion system. All the drugs used for specific treatments were added to the PSS and continuously perfused through the arterial bed.  

Experimental protocol. Three series of experiments were performed. The first series was conducted to determine the effects of MT-disrupting agents on vasodilator responses. For this purpose, the vascular beds were half-maximally preconstricted by phenylephrine, and dose-response curves to ACh (0.005–1.22 nmol), histamine (0.54–132.0 nmol), SNP (0.004–0.34 nmol), and pinacidil (1.0–40.0 nmol) were constructed. After this, phenylephrine perfusion was discontinued and nocodazole (10 μM), colchicine (30 μM), or vehicle [dimethyl sulfoxide (DMSO), 0.07% vol/vol] was perfused throughout the experiment. After 60 min of incubation with nocodazole, colchicine, or vehicle, the vascular beds were again preconstricted by phenylephrine and another dose-response curve to the same vasodilator agent was performed. In some vascular preparations the dose-response curves to histamine were constructed in a similar way, except that the vessels were continuously constricted with phenylephrine during nocodazole incubation. The concentration of phenylephrine perfused into the arteries was adjusted to prevent a potentiated constriction induced by phenylephrine after treatment with nocodazole or colchicine, as previously described (26, 38–40, 47), from having any significant influence on vasodilator responses.

In the second series of experiments, the possible mechanisms involved in the effect of MT-disrupting agents on the vasodilator response induced by histamine were investigated. To assess the role of endothelium, experiments were performed as described above, using vascular beds with no functional endothelium. In other vessels, the dose-response curves to histamine were constructed in the presence of pyrilamine (10 μM), ranitidine (30 μM) or N°-nitro-l-arginine methyl ester (l-NAME, 100 μM) before and after incubation with nocodazole.

Experiments in the third series were conducted to study the involvement of K⁺ channels in the effect of MT-disrupting drugs on the vasodilator response induced by histamine. For this, dose-response curves to histamine were constructed in mesenteric vessels half-maximally preconstricted with a modified high-K⁺ (50 mM) PSS solution before and after incubation with nocodazole. To investigate the involvement of K⁺ channels, the dose-response curves to histamine were performed in the presence of K⁺ channel blockers [10 mM tetraethylammonium (TEA), 1 μM dequalinium, and 1 μM glibenclamide] before and after incubation with nocodazole.

Drugs. Phenylephrine, ACh, histamine, SNP, colchicine, l-NAME, pinacidil, deoxycholic acid, dequalinium, glibenclamide, TEA, ranitidine, and pyrilamine were purchased from Sigma. Nocodazole was obtained from Fluka Chemie. Nocodazole and glibenclamide were diluted in DMSO (up to 20 mM). The final concentration of DMSO in the perfusion solution was 0.07%. All other drugs were diluted in PSS and prepared on the day of the experiment.

Data analysis. Changes in perfusion pressure are expressed as percentage of the maximal contractile response remaining. Values are expressed as means ± SE, and n = the number of rats. Differences between the mean values were compared with one-way ANOVA for repeated measures with post hoc Bonferroni’s test for multiple comparisons. Probabilities <5% (P < 0.05) were considered statistically significant.

RESULTS

Effects of nocodazole or colchicine on vasodilator responses. The results shown in Fig. 1 indicate that treatment with MT network-disrupting agents such as nocodazole or colchicine significantly attenuates the dose-dependent relaxation induced by histamine in the mesenteric arterial bed (Fig. 1, A and C, respectively). This effect was not different when the vascular beds were allowed to relax or were kept preconstricted with phenylephrine during the first 60 min of incubation with nocodazole (Fig. 1A). The vehicle (DMSO) did not affect the dose-response curve to histamine (Fig. 1B). Figure 1D shows that the vessels were stable throughout the experimental period.

Treatment of the mesenteric vessels with nocodazole did not change the relaxation induced by ACh, SNP, or pinacidil (Fig. 2, A, B, and C, respectively). No effect was observed after incubation with DMSO in the dose-response curves for the vasodilator agents mentioned above (data not shown).

Role of H₁ and H₂ receptors, NO, and endothelium on attenuation effect of nocodazole on histamine-induced relaxation. Blockade of H₁ receptors with pyrilamine significantly attenuated the relaxation induced by histamine in mesenteric vessels, and no further reduction was observed after incubation with nocodazole (Fig. 3A). On the other hand, blockade of H₂ receptors with ranitidine did not affect the histamine-induced relaxation significantly. Additional nocodazole treatment caused a significant decrease in the vasodilation induced by histamine in these vessels (Fig. 3B).

Perfusion of an endothelium-inactivating agent such as deoxycholic acid significantly reduced the vasodilation induced by histamine in the mesenteric arterial bed, and no further reduction of that response was observed after treatment with nocodazole (Fig. 4A). Histamine-mediated vasodilation was significantly attenuated by l-NAME, and no further reduction was observed after incubation with nocodazole (Fig. 4B).

Effect of high extracellular K⁺ and K⁺ channel blockers on attenuation effect of nocodazole on histamine-induced relaxation. High extracellular K⁺ significantly attenuated the relaxation induced by histamine in mesenteric vessels, and no further reduction was observed after treatment with nocodazole (Fig. 5A). The presence of K⁺ channel blockers such as TEA, dequalinium, and glibenclamide greatly reduced the histamine-mediated vasodilation in mesenteric arteries. However, additional incubation with nocodazole did not cause a significant change in the vasodilation induced by histamine in these vessels (Fig. 5B).

DISCUSSION

Novel mechanisms involved in alterations of vascular reactivity in isolated vessels have been of great interest to our laboratory (20). The aim of the present study was to evaluate the contribution of the MT network on the vasodilator response induced by histamine, ACh, SNP, and pinacidil in the rat isolated mesenteric arterial bed. The results showed that treatment with agents capable of disrupting the MT network, such as nocodazole or colchicine, significantly attenuated the vasodilation induced by histamine but, curiously, had no significant effect on the vasodilator response to ACh, SNP, and pinacidil.

Disruption of MTs with different agents has been shown to affect vascular reactivity. It potentiates receptor-mediated cal-
cium-dependent and -independent mechanisms involved in contractile responses. This effect could be explained in part by changes in the localization and/or activity of proteins or kinases such as phospholipase C, myosin light chain kinase (MLCK), and/or RhoA/Rho kinase pathway, thereby amplifying the cellular response to vasoconstrictor agonists (6, 20, 23, 26, 39–41, 47). On the other hand, MT disruption decreases vasodilator responses to flow but has no effect on the vasodilator response induced by agonists such as ACh and SNP (19, 50). It has also been reported that colcemid and vinblastine treatment attenuates endothelium-independent adenosine-induced vasodilation responses in rat skeletal muscle arterioles (40).

The concentration of the two pharmacological agents used in this study to shift the dynamic state of MTs toward depolymerization, nocodazole and colchicine (10 and 30 μM, respectively), were tested previously in different experiments by our group (5, 20, 26). Considerable published data with immunofluorescence and confocal microscopy techniques have confirmed that concentrations of these agents ranging from 0.5 to 33 μM are capable of preventing assembly of tubulin subunits in endothelial and vascular smooth muscle cells after 60-min perfusion of vessels or incubation of cultured cells (22, 30, 46, 50).

Histamine induces a dose-dependent fall in arterial pressure and is present at significant concentrations in blood and in vascular tissues. Many studies have reported that the vasodilator effects are likely to be mediated indirectly through the activation of H₁ and/or H₂ receptors, with consequent release of mediators including NO, prostacyclin (PGI₂), and endothelium-derived hyperpolarizing factor (EDHF) acting directly or indirectly through increase in levels of intracellular cGMP or cAMP (7, 27, 28). However, the precise pathways involved in the response to histamine in vitro are controversial, varying in different physiological situations, in different vessels, and in different species (1, 15, 53–55).

In the mesenteric arterial bed, histamine elicits relaxation through an activation of histamine H₁ receptors dependent on an intact endothelium (24, 55). Histamine can also induce relaxation of the mesenteric vessels through the activation of histamine H₂ receptors within smooth muscle cells (10).

Our results confirmed that histamine induces a dose-dependent dilation of rat isolated mesenteric arterial bed and that its vasodilator effect was significantly attenuated by treatment with an H₁ but not an H₂ receptor antagonist, 1-NAME, K⁺ channel blocker cocktail, or high extracellular K⁺ or by the absence of a functional endothelium. Therefore, we conclude that histamine dilates mesenteric vessels predominantly through activation of H₁ receptors in the endothelial cells and that the response is mainly mediated by hyperpolarization of the vascular smooth muscle cells via a NO-dependent mechanism.
MT disruption with nocodazole or colchicine significantly attenuated histamine-mediated vasodilation in mesenteric vessels. Perfusion of nocodazole in vessels pretreated with the H1 receptor antagonist pyrilamine, L-NAME, K+ channel blocker cocktail, high extracellular K+, or deoxycholic acid did not induce an additional attenuation of the dilation elicited by histamine.

It was previously demonstrated (30) that MT disruption with nocodazole decreases inducible endothelial NO synthase (eNOS) expression in cultured aortic cells but does not change the expression of the constitutive enzyme. Conversely, it was also demonstrated that MT disruption increases NO production in human umbilical vein (22). These observations do not explain the attenuation of the histamine-induced vasodilator response after MT disruption observed in our study.

It has been demonstrated that treatment with nocodazole abolishes flow-dependent dilation of arterioles from rat skeletal muscle (50) and rabbit aorta segments (19). Both studies suggested an inhibition of NO release following a MT disruption because this effect mimics that observed with the treatment with L-NAME (19). In the same studies, it was observed that MT disruption did not alter the vasodilator effect of agonists such as ACh, SNP, prostaglandin E2, or arachidonic acid.

Fig. 2. Dose-response curves to acetylcholine (ACh; A), sodium nitroprusside (SNP; B), or pinacidil (C) in perfused rat mesenteric arterial bed. The vessels were preconstricted with phenylephrine (15–30 μM), and the curves to ACh (0.005–1.22 nmol), SNP (0.004–0.34 nmol), or pinacidil (1.0–40.0 nmol) were constructed before and after 60-min incubation with nocodazole (10 μM).

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Fig. 3. Dose-response curve to histamine in perfused rat mesenteric arterial bed. The vessels were preincubated for 30 min with 10 μM pyrilamine (A) or 30 μM ranitidine (B) and constricted with phenylephrine (15–30 μM). The curves to histamine (0.54–132.0 nmol) were constructed before and after 60-min incubation with nocodazole (10 μM). The control curves for histamine are shown for comparison. *P < 0.05 compared with respective control (1-way ANOVA followed by Bonferroni’s post hoc test).
Another study showed that incubation of pulmonary artery endothelial cells with nocodazole significantly decreased NO production, eNOS activity, and the amount of heat shock protein (HSP)90 binding to eNOS (49).

The results shown here confirm that ACh, SNP, and pinacidil induce a dose-dependent dilation of the rat mesenteric arterial bed. They also demonstrate that treatment of these vessels with nocodazole did not change the dilation induced by ACh, SNP, or pinacidil.

Pinacidil, an endothelium-independent vasodilator, causes hyperpolarization by opening ATP-sensitive K⁺ channels (17). SNP, a NO donor, relaxes smooth muscle cells independent of endothelium through an increase in intracellular levels of cGMP, opening calcium-dependent K⁺ channels via activation of protein kinase. In some arteries, NO causes relaxation at lower concentrations without hyperpolarization, although at higher concentration hyperpolarization is always observed (34, 52). Because the SNP and pinacidil responses were not affected by nocodazole, one could conclude that MT disruption does not significantly affect the NO downstream effectors.

In a variety of blood vessels, ACh causes relaxation of smooth muscle cells indirectly by releasing some vasorelaxing mediators from the endothelium through activation of M₃ muscarinic receptors. These mediators, depending on the vessel type, may include NO, PGI₂, and EDHF (9, 12, 51). In the mesenteric bed, ACh elicits a relaxation that is sensitive to K⁺ channel blockers but not significantly affected by NOS inhibitors (2, 13, 31, 37, 43, 57), suggesting that the vasodilation induced by ACh-mediated hyperpolarization is not totally dependent on the NO-cGMP pathway.

The effect of nocodazole observed in this study seems to be specific because it has been shown to be reversed by paclitaxel, which is known to cause an increased assembly of stable microtubules (45), either in terms of potentiation of the vasoconstrictor response induced by phenylephrine in mesenteric arterial bed. The vessels were preconstricted with phenylephrine (15–30 μM), and the curves to histamine (0.54–132.0 nmol) were constructed before and after incubation with nocodazole in vessels treated with deoxycholic acid to inactivate endothelium (E⁻; A) or in vessels treated with 100 μM N⁵-nitro-L-arginine methyl ester (L-NAME; B). The control curves for histamine are shown for comparison. *P < 0.05 compared with respective control (1-way ANOVA followed by Bonferroni’s post hoc test).

In B, the vessels were preincubated for 30 min with 10 mM tetraethylammonium (TEA), 1 μM dequalinium (Deq), and 1 μM glibenclamide (Glib) and constricted with phenylephrine (15–30 μM). The curves to histamine (0.54–132.0 nmol) were constructed before and after 60-min incubation with nocodazole. The control curves for histamine are shown for comparison. *P < 0.05 compared with respective control (1-way ANOVA followed by Bonferroni’s post hoc test).

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vessels (26) or in terms of attenuation of the histamine-induced relaxation of rat isolated tail artery rings (unpublished data from our laboratory). Although the possibility that nocodazole itself could affect binding of histamine to H1 receptors cannot be ruled out, it seems very unlikely. There have been no reports on nocodazole H1 receptor antagonist properties, it shows no structural similarity with H1 antihistamine drugs, and furthermore, colchicine, which has a different structure, shows the same effect on attenuating histamine vasodilator responses.

Potentiation of vascular smooth muscle constriction after treatment with MT-disrupting agent was also observed in our studies as described previously (26, 38–40, 47). This enhanced vasoconstrictor tone could have affected the agonist-induced vasodilation, but this does not seem to be the case in the present study, first because the concentration of phenylephrine was adjusted to prevent a potentiated constrictor effect after incubation with nocodazole or colchicine and second because the vasodilation mediated by ACh, SNP, and pinacidil was not significantly affected by nocodazole treatment. This does not necessarily mean, however, that the state of MT assembly in the smooth muscle was not changed by nocodazole or colchicine.

Together, these results suggest that MT disruption affects an essential endothelial component of histamine-mediated vasodilation in the mesenteric arterial bed. We hypothesize that the mechanism(s) involved in the attenuation of histamine-mediated vasodilation by MT disruption could be related to an impairment of endothelial NO synthesis, which might not be the most important mechanism for the ACh response in mesenteric vessels.

Whether eNOS activity per se is reduced in the mesenteric arterial bed is hard to assess with the data from this study. On the other hand, in another study from our laboratory (unpublished data), nocodazole treatment did not change the endothelium-dependent receptor-independent mediated relaxation induced by calcium ionophore A-23187 in rat isolated tail artery ring. On the basis of this finding one could suggest that MT disruption does not affect the ability of eNOS to produce NO. However, the well-established mechanism for A-23187 relaxation-dependent eNOS-Ca2+/calmodulin-NO formation has been challenged by some evidence indicating that in resistance vessels the relaxation to A-23187 involves NO-independent hyperpolarization as well (35–37).

Inflammatory mediators such as thrombin, bradykinin, and particularly histamine increase endothelial permeability accompanied by reversible cell rounding and interendothelial gap formation (11). This increase in permeability is mainly due to contraction (MLCK-dependent mechanism) or passive retraction (change in the cell-cell and cell-matrix contacts) of the endothelial cells (3, 29, 33). These changes in endothelial cell structure and shape, probably due to changes in the F-actin microfilaments induced by histamine, might have a potentiation effect on the attenuation of histamine-induced vasodilation in the presence of MT-disrupting agents such as nocodazole or colchicine (29).

The enzyme eNOS is found in the plasmalemmal caveolae, invaginations in the endothelial cell plasma membrane that are characterized by the presence of caveolin. The caveolin-eNOS association serves to inhibit or suppress the catalytic activity of eNOS (21, 44). Increasing vascular flow or pressure in situ rapidly activates caveolar eNOS, with apparent eNOS dissociation from caveolin and association with calmodulin, leading to an increase in NO production (44).

Therefore, we speculate that disruption of MTs in endothelial cells could impair the association of eNOS with important intracellular signaling proteins such as HSP90 and/or calmodulin, because the existence of a possible role for a close association between those proteins in many different cells has been suggested (14, 48, 49, 58). This impairment would lead to a decrease in NO synthesis and, consequently, to an attenuated vasodilator response induced by histamine.

There have been some reports from studies of neuronal tissue showing that accumulation of free tubulin-GTP monomers after MT network depolymerization can interact directly with G proteins to influence the adenylate cyclase pathway (42, 56). Whether an excess of free tubulin-GTP monomers interacts with G proteins or other signaling elements in the endothelial cells to, for example, inhibit inositol 1,4,5-trisphosphate formation, impair Ca2+ mobilization from intracellular stores, and consequently reduce agonist-induced NO production remains to be determined.

The results presented here highlight the importance of the microtubular system for endothelium-dependent NO-mediated smooth muscle relaxation. The disruption of MTs with nocodazole and colchicine attenuated the vasodilation to histamine but did not affect the responses elicited by other vasodilators such as ACh, SNP, and pinacidil in the rat isolated mesenteric arterial bed.

GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grant HL-74167 and grants from Fundação de Amparo à Pesquisa do Estado de Minas Gerais, Conselho Nacional de Desenvolvimento Científico e Tecnológico, and Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Brazil).

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