

Farnesyl Analogues Inhibit Vasoconstriction in Animal and Human Arteries

Jean-Baptiste Roulet,* Hong Xue,* Justin Chapman,* Patrick McDougal,[‡] Chantal M. Roulet,* and David A. McCarron*

*Department of Nephrology, Hypertension and Clinical Pharmacology, Oregon Health Sciences University, Portland, Oregon 97201; and [‡]Chemistry Department, Reed College, Portland, Oregon 97202

Abstract

Recent studies have suggested that nonsterol, mevalonate-derived metabolites are implicated in the control of vascular tone and blood pressure. Because of the metabolic importance of farnesyl pyrophosphate, a 15-carbon (C₁₅) intermediate of the cholesterol pathway, the vasoactive properties of the farnesyl motif were investigated. Two farnesyl analogues were used: farnesol, the natural dephosphorylated form of farnesyl pyrophosphate, and *N*-acetyl-*S*-*trans,trans*-farnesyl-L-cysteine (AFC), a synthetic mimic of the carboxyl terminus of farnesylated proteins. Both compounds inhibited NE-induced vasoconstriction in rat aortic rings at micromolar concentration. Their action was rapid, dose dependent, and reversible. Shorter (C₁₀) and longer (C₂₀) isoprenols as well as *N*-acetyl-*S*-geranyl-L-cysteine (C₁₀) did not inhibit the response to NE. In contrast, *N*-acetyl-*S*-geranylgeranyl-L-cysteine (C₂₀), exhibited vasoactive properties similar to AFC. It was further demonstrated that AFC and farnesol inhibited KCl and NaF-induced contractions, suggesting a complex action on Ca²⁺ channels and G protein-dependent pathways. Finally, the effect of farnesol and AFC on the NE response was reproduced in human resistance arteries. In conclusion, mevalonate-derived farnesyl analogues are potent inhibitors of vasoconstriction. The study suggests that farnesyl cellular availability is an important determinant of vascular tone in animals and humans, and provides a basis for exploring farnesyl metabolism in humans with compromised vascular function as well as for using farnesyl analogues as regulators of arterial tone in vivo. (*J. Clin. Invest.* 1996. 97:2384–2390.) Key words: isoprenoids • vascular tone • farnesol • G proteins • farnesylation

Introduction

The impact of cholesterol on the vascular wall has received considerable attention because excess cholesterol is atherogenic and thereby affects vascular tone. Several reports have

now demonstrated that hypercholesterolemia increases the vascular response to vasoconstrictors such as NE and impairs vasodilation in large and small arteries (1, 2). By increasing peripheral vascular resistance, cholesterol may contribute to elevating arterial pressure and account for the frequently observed association between hypercholesterolemia and hypertension in humans (3–5).

Much less is known about cholesterol precursors and their role in vascular physiology. In two recent studies (6, 7), we observed that systemic blood pressure and vascular tone were dependent on the metabolic availability of mevalonate, an early precursor of cholesterol. Reduction of mevalonate availability with lovastatin, a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA)¹ reductase inhibitor, enhanced the response of conductance and resistance vessels to vasoconstrictors, impaired vasodilation, and raised arterial pressure (6). Increased mevalonate availability with exogenous mevalonate had opposite effects and normalized the reactivity of vessels exposed to lovastatin in vitro. We further demonstrated that mevalonate availability affected vascular contractility by maintaining appropriate intravascular free Ca²⁺ levels and established the regulatory role of mevalonate on vascular tone in human resistance arteries (7). Because the cardiovascular action of mevalonate was not associated with any significant change in tissue or plasma cholesterol levels, the data suggested that metabolites derived from mevalonate other than cholesterol were responsible for the control of vascular tone and potentially of arterial pressure.

Farnesyl pyrophosphate (FPP) is one of several nonsterol mevalonate derivatives. It is a 15-carbon (C₁₅) isoprenoid lipid with geranyl-PP (C₁₀) as its immediate precursor. FPP can be either dephosphorylated into farnesol, transformed into geranylgeranyl-PP (C₂₀, reference 8), or covalently attached (farnesylation) to a cysteine residue at the carboxyl terminus of several cellular proteins implicated in cell signaling (9, 10). Farnesylation is followed by amino acid cleavage and carboxyl methylation of the exposed (COOH terminal) farnesylated cysteine (11). Two recent studies demonstrated that farnesol and FPP analogues were potent HMG-CoA reductase inhibitors (12, 13). Other studies have established that protein farnesylation is crucial to the control of receptor-activated signal transduction in mammalian cells. In particular, it is believed that the farnesyl cysteine motif represents a key for the activation of downstream intracellular effectors of G proteins and for G protein membrane targeting (14–16). Altogether, these

Address correspondence to Jean-Baptiste Roulet, Ph.D., Department of Nephrology, Hypertension and Clinical Pharmacology, Oregon Health Sciences University, 3181 SW Sam Jackson Park Road, Portland, OR 97201. Phone: 503-494-4979; FAX: 503-494-6725.

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1. Abbreviations used in this paper: Ach, acetylcholine; AFC, *N*-acetyl-*S*-*trans,trans*-farnesyl-L-cysteine; AGC, *N*-acetyl-*S*-*trans,trans*-geranyl-L-cysteine; AGGC, *N*-acetyl-*S*-all-*trans*-geranylgeranyl-L-cysteine; FPP, farnesyl pyrophosphate; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; NAC, *N*-acetyl-L-cysteine.

reports underscore the metabolic and functional importance of farnesyl in cell biology and suggest that farnesyl, either as farnesol or as farnesyl cysteine, could be a mediator of the mevalonate-dependent regulation of vascular tone reported previously by us.

Therefore, we sought to characterize the vasoactive properties of this motif using farnesol, the naturally occurring diphosphorylated form of FPP, and *N*-acetyl-*S*-*trans,trans*-farnesyl-L-cysteine (AFC), a synthetic mimic of the carboxyl terminus of farnesylated proteins. Isoprenoids with shorter (C_{10}) or longer (C_{20}) hydrocarbon motifs were also tested to determine structure-activity relationships and establish the functional importance of farnesyl among other isoprenoids.

Methods

Reagents. C_{15} (farnesol), C_{10} (geraniol), and C_{20} (geranylgeraniol) isoprenols were purchased from Aldrich Chemical Co. (Milwaukee, WI) (*trans-trans*-farnesol, catalog No. 27,754-1) and Sigma Chemical Co. (St. Louis, MO) (*trans* geraniol, product No. G 5135, and all-*trans* geranylgeraniol, product No. G 3278). AFC and *N*-acetyl-*S*-*trans,trans*-geranyl-L-cysteine (AGC) were prepared (purity < 99%) by incubation of *trans,trans*-farnesyl bromide (for AFC) or geranyl bromide (for AGC) with *N*-acetyl-L-cysteine (NAC) and characterized by nuclear magnetic resonance and mass spectroscopy (17). *N*-acetyl-*S*-all-*trans*-geranylgeranyl-L-cysteine (AGGC) was obtained from Calbiochem (San Diego, CA). The stock solutions of isoprenols and *N*-acetyl-*S*-isoprenyl-L-cysteine analogues were prepared in ethanol. Other chemicals were from Sigma Chemical Co.

Rat aortic ring experiments. Thoracic aortas were isolated from 12–14-wk-old male Wistar rats (Charles River Breeding Laboratories, Inc., Boston, MA). Three to six rings per animal were prepared. They were mounted in a 6-channel muscle bath apparatus (18) and incubated in a physiological saline solution (6) containing variable concentrations of isoprenoids for the indicated period of time (see Results). Ethanol ($\leq 0.1\%$, vol/vol) was used as vehicle and control. The response to vasoconstrictors was then determined, using either NE (10^{-9} – 10^{-5} M), KCl (10–100 mM), or NaF (10 mM). $AlCl_3$ (0.5 mM) was used to enhance fluoride-induced contraction (19, 20). The force generated after addition of each agonist (KCl, NE, and NaF) was normalized by the cross-sectional area of the vessels (active force development in N/m^2 , reference 18). Relaxation was also studied us-

ing acetylcholine (ACh, 10^{-8} – 3×10^{-5} M) after contraction with NE (6) and expressed as percentage of precontraction with NE (100% = no relaxation). ED_{50} were either graphically evaluated from plots of agonist concentration versus percentage of maximal response (KCl) or calculated (NE, ACh) by computerized nonlinear curve fitting (Statgraphics® Plus; Manugistics, Inc., Rockville, MA).

Human resistance vessel experiments. Human subjects were recruited in the surgery department of our institution. Biopsies of subcutaneous fat were performed after informed consent from the patients. The protocol was approved by the Oregon Health Sciences University Institutional Review Board. Arterial segments (~ 200 μ m diameter, 1–3 mm length) were carefully dissected, mounted in a perfusion myograph (Living Systems Instrumentation, Burlington, VT), and set at a constant pressure of 50 mmHg. Each vessel served as its own control, the effect of vehicle (ethanol) tested first, and that of farnesol or AFC tested second. All substances and agonists (NE) were applied extraluminally. Changes in lumen diameter were determined by video imaging and expressed as percentage of the greatest change obtained with NE in the presence of vehicle (21). There was no apparent relationship between the patients' personal history and their medications (all were admitted for peripheral vascular diseases) and the response to either one of the tested compounds (not shown).

Statistical analysis. Values are reported as mean \pm SEM. Differences were assessed using paired tests (Student's *t* test or Wilcoxon signed rank test as appropriate) and a *P* value < 0.05 was assumed to indicate a significant difference.

Results

Effect of farnesol and related isoprenols on NE-induced contraction (rat aortic rings). Farnesol (0–30 μ M) had a profound dose-dependent inhibitory action on NE-induced contraction, affecting both maximum response ($NE^{E_{max}}$) and sensitivity. After a 30-min incubation, NE- ED_{50} were (nM): 19.7 ± 2.6 ($n = 6$) for control, 63.7 ± 15.1 for 15 μ M farnesol ($P < 0.04$ vs. control), and 306.9 ± 185.1 for 30 μ M farnesol ($P < 0.03$ vs. control). The effect was rapid (significant after 10 min, Fig. 1A) and time dependent; after a 1-h incubation, the response to NE was essentially abolished (Fig. 1B). Normal response to NE was restored after several washes of the vessels, indicating a reversible mechanism of action of the compound. In contrast to farnesol, geraniol (0–60 μ M, 30-min incubation) had no sig-

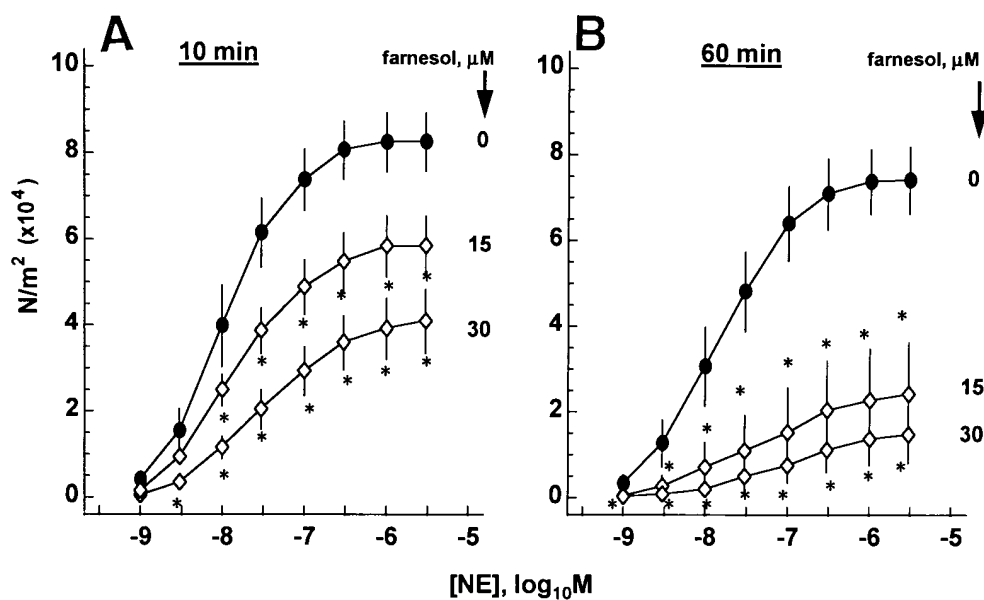


Figure 1. Effect of farnesol on NE-induced contraction. Rat aortic rings (three segments per animal) were prepared and incubated with the indicated concentrations of farnesol for 10 min (A) or 60 min (B). Asterisks indicate a significant difference with control (no farnesol) vessels ($n = 5$ independent experiments).

nificant inhibitory effect on NE-induced contraction (Table I). In these conditions, geranylgeraniol also did not inhibit the NE response (Table I). Further, a moderate potentiation of this response (decrease of NE-ED₅₀ without change in NE^{max}) was observed at 30 μM geranylgeraniol (not 60 μM) when compared with controls. Longer incubation (60 min) did not reveal any late effects on the response of NE (data not shown).

Effect of AFC and acetyl-isoprenyl cysteine analogues on NE-induced contraction (rat aortic rings). AFC (0–60 μM) inhibited NE-induced contractions of aortic rings, decreasing maximum contraction as well as sensitivity to the hormone (Fig. 2). The effect of AFC, although of smaller magnitude than that of farnesol (see Fig. 1 B), was nonetheless dose and time dependent. An increase of ~ 300% in NE-ED₅₀ (nM) was observed after only 10 min of incubation with 60 μM AFC (139.6±53.2 AFC vs. 49.0±23 control, *P* < 0.04, *n* = 6, paired *t* test) and reached ~ 600% after 60 min (248.4±39.3 AFC vs 38.1±7.9 control, *P* < 0.002). As observed with farnesol, normal response to NE was restored after several washes, indicating a reversible mechanism of action of the compound. Structure–activity relationship was next explored. NAC had no significant effect on the response to NE. NE^{max} (in N/m² [×10⁴]) were 6.3±1.2, 7.5±1.8, and 7.3±1.0 for, respectively, 0, 30, and 60 μM NAC, whereas NE-ED₅₀ (nM) were 18.9±4.7, 16.6±5.5, and 20.1±8.5 (60-min incubation, *n* = 3, *P* = NS). These results indicate that the presence of an isoprenyl chain is necessary to confer biological activity to the acetyl cysteine motif. The isoprenyl length was then varied. AGC, a shorter (C₁₀) farnesyl cysteine analogue, was inactive (Fig. 3 B). In contrast, AGGC, a longer (C₂₀) analogue, strongly inhibited NE-induced vasoconstriction (Fig. 3 A).

Effect of farnesol and AFC on KCl-induced contraction (rat aortic rings). The response to KCl was inhibited by farnesol in a time- and dose-dependent manner (Table II). AFC also inhibited KCl-induced contraction (Fig. 4), although, on a molar basis, the effect was of smaller magnitude than that of farnesol: after 1 h of incubation, the decrease in KCl^{max} reached ~ 70% of the control value for 30 μM farnesol and only ~ 37% for 30 μM AFC, whereas ED₅₀ were increased by 50 and 25%, respectively (Table I and Fig. 4).

Effect of farnesol and AFC on NaF-induced contraction (rat aortic rings). Several studies suggest that fluoride, when associated with aluminum, mimics the γ-phosphate of GTP and can directly activate G proteins (22, 23). Therefore, to determine if the effects of farnesol and AFC were at the receptor (NE) versus G protein levels, NaF experiments were per-

Table I. Effect of Geraniol and Geranylgeraniol on NE-induced Contraction in Rat Aortic Rings (30-min Incubation)

		Isoprenol concentration (μM)		
		0	30	60
Geraniol (<i>n</i> = 6)	NE ^{max}	7.4±0.3	9.1±1.3	7.7±0.1
	ED ₅₀	16.4±3.1	14.7±8.2	18.9±6.5
Geranylgeraniol (<i>n</i> = 7)	NE ^{max}	7.0±0.2	7.8±0.6	7.6±0.3
	ED ₅₀	21.5±4.3	13.9±2.4*	16.2±3.1

Maximum response to NE, NE^{max}, is expressed in N/m² [× 10⁴]. ED₅₀ in nM. Values are mean±SEM (number of independent experiments in parentheses). *Significant difference (*P* < 0.05) with control.

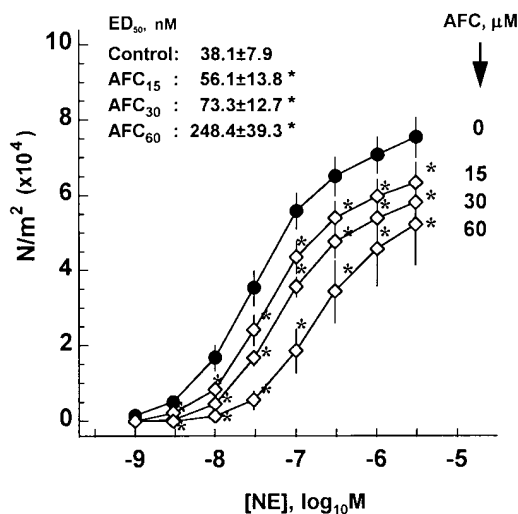


Figure 2. Effect of AFC on NE-induced contraction. Rat aortic rings (four segments per animal) were incubated with the indicated concentrations of AFC for 60 min. Asterisks indicate a significant difference with control (no AFC) vessels (*n* = 6 independent experiments).

formed. Both compounds strongly inhibited the response to fluoride (Fig. 5). The effect was concentration dependent and of greater magnitude for farnesol than for AFC.

Effect of farnesol and AFC on vasodilation (rat aortic rings). Typically, the response to vasodilators such as Ach is determined after precontraction of the aortic rings (with NE). To allow accurate and reliable determination of the response, precontraction must be stable and of sufficient amplitude. With farnesol, these optimal conditions could not be reliably achieved. Some preparations spontaneously relaxed after NE addition; in others, contraction was completely abolished (not shown). On average, tone gradually declined over time even for concentrations as low as 15 μM (see Fig. 1, B vs. A). Thus, the effect of farnesol on aortic ring relaxation was not evaluated. In contrast, optimal precontraction conditions could be achieved with relatively low concentrations of AFC (30 μM) and only 30 min of incubation. Therefore, the effect of AFC on relaxation was determined. The results of these experiments showed that the response to Ach was inhibited by AFC. Ach^{max} (percentage of precontraction with NE) were 57.1±14.1 and 27.1±11.2 for AFC and control, respectively (*n* = 7, *P* < 0.01), whereas Ach-ED₅₀ (nM) were 638.3±230.0 and 190.6±36.1 (*P* < 0.02).

Effect of farnesol and AFC on NE-induced contraction (human arteries). As illustrated in Fig. 6, both compounds significantly reduced the response to NE. Maximum constriction was decreased 19.1±3.4% by AFC (30 μM, 30-min incubation, *P* < 0.001) and 34.6±5.8% by farnesol (30 μM, 30-min incubation, *P* < 0.001) compared with control, whereas NE-ED₅₀ was increased approximately twofold (Fig. 7). Finally, ~ 2 min after addition, each farnesyl analogue increased significantly the inner diameter of the arteries in the absence of added NE (resting diameter): 5.2±1.1% (*P* < 0.002, *n* = 11) for AFC, and 3.7±0.4% (*P* < 0.001, *n* = 8) for farnesol.

Discussion

In this study, we explored the vascular function of farnesyl, a key intermediate of the mevalonate pathway, and identified

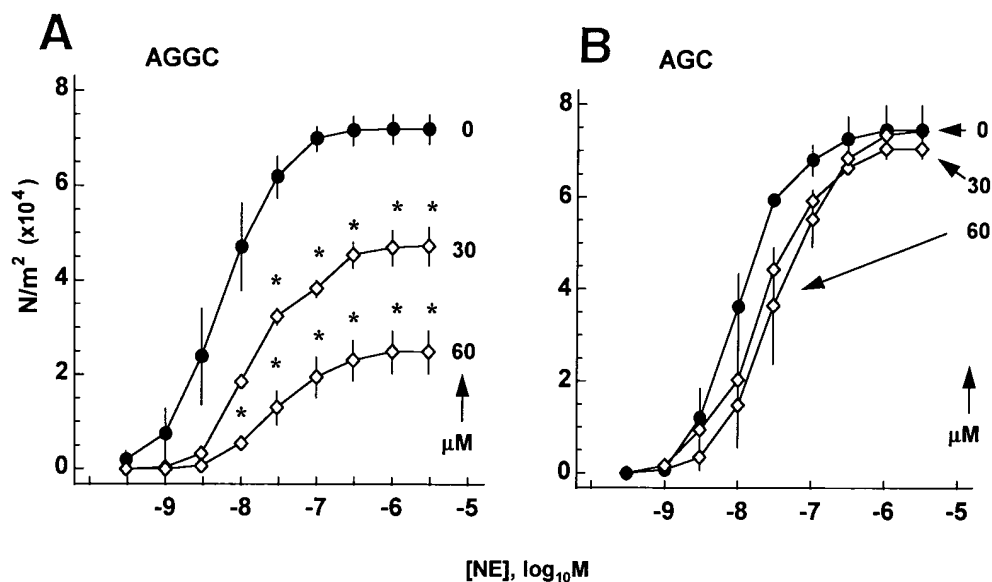


Figure 3. Effect of AGGC (A) and AGC (B) on NE-induced vasoconstriction (rat aortic rings). Vessels (three segments per animal) were incubated with either ethanol (vehicle) or isoprenyl cysteine analogues (30 and 60 μM) for 60 min. Asterisks indicate a significant difference with control vessels ($n = 3$ independent experiments).

several farnesyl analogues with previously unrecognized potent vasoactive properties.

In a first series of experiments, the action of farnesol was characterized and compared with that of geraniol (C_{10}) and geranylgeraniol (C_{20}). These isoprenols have been used *in vitro*, at micromolar concentration, to restore cell growth after blockade with lovastatin (24–26), suggesting that they can be phosphorylated and reincorporated in the mevalonate pathway *in vivo*. Other data in cultured cells have shown they can serve as substrate for protein prenylation reactions (27). Our experiments conducted with rat aortic rings indicate that farnesol is a powerful, reversible inhibitor of NE-induced vasoconstriction. To our knowledge, the vascular action of farnesol has not been reported previously. Because farnesol is a normal component of the mevalonate pathway, the data suggest that the C_{15} isoprenoid is a potent antagonist of vasoconstriction *in vivo*. The compound is likely to be taken up by intact vessels as demonstrated for cells in culture. However, it is difficult from the present experiments to evaluate the actual cellular site of action as well as the active intracellular concentration. Farnesol action is rapid but time dependent (Fig. 1). This may represent metabolic activation, diffusion through the plasma mem-

brane, or both. Of interest is the reversibility of the farnesol action: not only does it confer to the compound pharmacologically useful properties, but it raises the possibility that farnesol-specific receptors are present in arteries.

As farnesol and FPP derive from mevalonate, our results also provide an explanation for our previous findings on the regulation of vascular tone by mevalonate availability (see Introduction and references 6 and 7). In the presence of lovastatin, the production of FPP (or of farnesol if FPP needs to be dephosphorylated to be active) would decrease and vascular tone would increase, whereas, in the presence of excess mevalonate, FPP production would be stimulated and the response to vasoconstriction would be attenuated. Although these hypotheses need confirmation by direct measurement of the vascular levels of FPP or farnesol during HMG-CoA reductase inhibition, our past and present results suggest that the metabolic availability of the farnesyl motif participates actively to the maintenance of vascular tone.

Table II. Effect of Farnesol on KCl-induced Contraction in Rat Aortic Rings

Incubation time	Response to KCl	[Farnesol]		
		0 μM	15 μM	30 μM
10 min	KCl ^{max}	5.9±0.6	4.8±0.5*	3.6±0.3*
	ED ₅₀	24.6±1.2	29.2±0.9*	27.4±0.6*
30 min	KCl ^{max}	5.3±0.5	2.8±0.3*	2.1±0.3*
	ED ₅₀	26.4±0.9	39.5±1.1*	53.3±6.2*
60 min	KCl ^{max}	4.71±0.3	2.4±0.3*	1.5±0.4*
	ED ₅₀	27.5±1.4	40.8±1.7*	52.1±8.6*

Maximum response to KCl, KCl^{max}, is expressed in $\text{N}/\text{m}^2 [\times 10^4]$, ED₅₀, in mM. Values are mean±SEM ($n = 6$ independent experiments). *Significant difference ($P < 0.05$) with time-matched control (no farnesol).

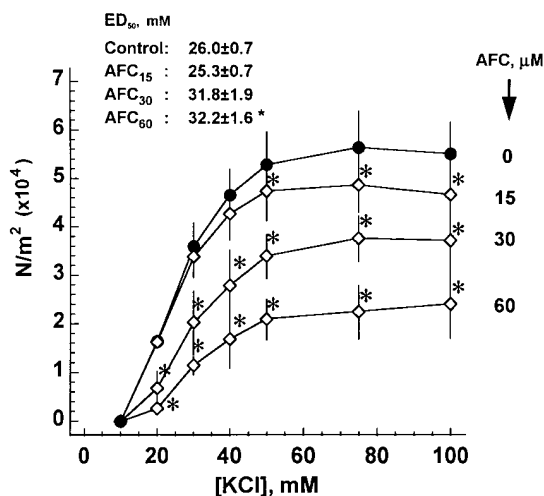


Figure 4. Effect of AFC on KCl-induced contraction. Rat aortic rings (four segments per animal) were incubated with the indicated concentrations of AFC for 60 min. Asterisks indicate a significant difference with control (no AFC) vessels ($n = 5$ independent experiments).

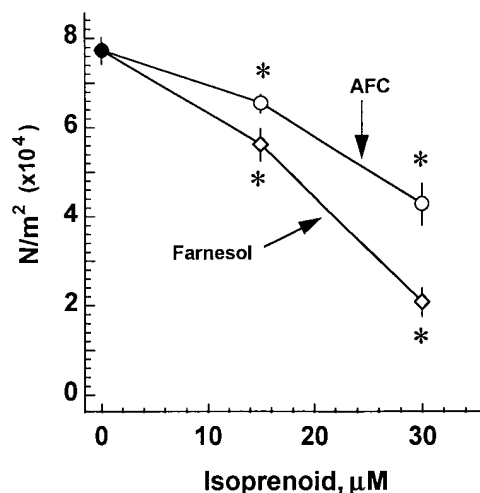


Figure 5. Effect of farnesol and AFC on NaF-induced vasoconstriction. Rat aortic rings (three segments per animal) were incubated with either ethanol or isoprenoid (15 and 30 μM) for 30 min, and with AlCl_3 (0.5 mM) for 15 min. NaF was then added (10 mM) to initiate contraction. Asterisks indicate a significant difference with vehicle ($n = 3$ and 4 independent experiments for farnesol and AFC, respectively).

The strong inhibitory properties of farnesol appear to be specific of the C_{15} structure as geraniol (C_{10}) and geranylgeraniol (C_{20}) do not inhibit the response to NE (a small activation of this response was actually observed with low concentrations of geranylgeraniol). A similar structure-activity relationship was reported by Correll et al. (13). Using the met-18b-2 cell line, these authors demonstrated that either farnesol or FPP after dephosphorylation, but not geraniol or geranylgeraniol, accelerated the degradation of HMG-CoA reductase at micromolar concentrations; as in our experiments, the farnesol action was rapid and dose dependent. It is interesting to note that the farnesol concentrations which inhibit vascular contraction are very similar to the ones effective in degrading HMG-

CoA reductase (9). They are also similar to the Michaelis constant (K_m) of the microsomal *cis*-prenyltransferase (geranylgeranyl synthase) which synthesizes geranylgeranyl-PP from FPP ($\sim 25 \mu\text{M}$, in reference 28). Thus, the micromolar concentrations of farnesol used in our study might be physiologically relevant. Altogether, these observations confirm the importance of farnesol in cell function and establish its importance in vascular physiology.

In a second series of experiments, we evaluated the vasoactive properties of AFC and found that it inhibits agonist-induced vasoconstriction. As observed with farnesol, the effect was rapid and reversible, although of less potency on a molar basis. Similar to the farnesyl cysteine motif of cellular G proteins, AFC is recognized by intracellular prenylcysteine methyltransferase and methylated (29, 30). The K_m of the enzyme for AFC is $\sim 20 \mu\text{M}$ (17, 30), which is close to the concentration of AFC necessary to double NE-ED₅₀ (30 μM , see NE-ED₅₀ in Fig. 2). It has been further demonstrated that AFC could block signal transduction in nonvascular cells or tissues, presumably by inhibiting G protein carboxyl methylation (30–33). Therefore, our results suggest that AFC is actually taken up by the arteries and reaches intravascular concentrations compatible with an inhibition of vascular methyltransferase as demonstrated in other tissues (30, 33). They also imply that G protein carboxyl methylation is implicated in vascular signal transduction. This hypothesis is further supported by our experiments with *N*-acetyl-*S*-isoprenyl-L-cysteine analogues, AGC (C_{10}) and AGGC (C_{20}), and with NAC, the parent compound used for the synthesis of AFC. Neither NAC nor AGC had any significant impact on vascular contraction. This was expected as both compounds, unlike AFC, are poor substrates and weak inhibitors of protein carboxyl methyltransferase (29, 33, 34). In contrast, AGGC exhibited a significant inhibitory action on vasoconstriction, which, at equimolar concentration, was even stronger than that observed with AFC (Figs. 2 and 3). A similar order of potency was reported by Philips et al. (33) in their study of the role of protein carboxyl methylation during signal transduction in neutrophils and could be explained by the low K_m of AGGC for carboxyl methyltrans-

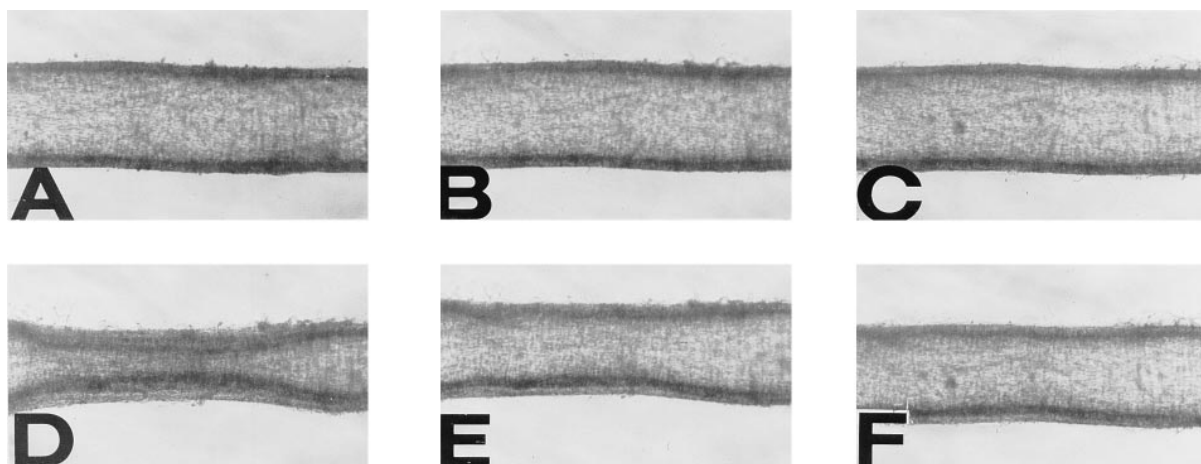


Figure 6. Effect of farnesol and AFC on NE-induced contraction in human resistance arteries. (A) Resting control (ethanol); (B) resting AFC (60 μM , 30-min incubation); (C) resting farnesol (60 μM , 30-min incubation); (D) control + NE (10^{-6}M); (E) AFC + NE; (F) farnesol + NE. Photographs were taken with a Nikon inverted microscope, equipped with a $\times 20$ lens. The resting inner diameter of the vessel (A) was 185 μm . The same vessel was used for all conditions, ethanol being tested first, AFC second, and farnesol last. Several washes and verification that full constriction to NE was restored were performed before testing farnesol.

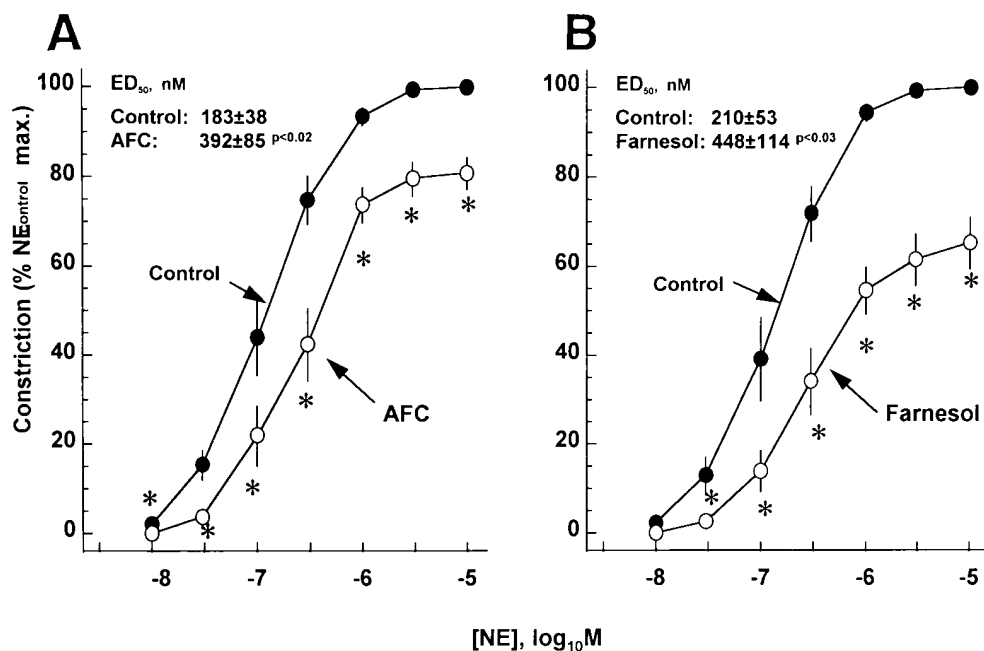


Figure 7. Effect of AFC and farnesol (30 μ M, 30-min incubation) on NE-induced contraction in human resistance arteries. Each vessel served as its own control, the effect of vehicle (ethanol) tested first and that of farnesol (B) or AFC (A) tested second. Y-axis represents constriction expressed as percentage of the greatest change in lumen diameter obtained with NE in control vessels. Mean (\pm SEM) resting control lumen diameters were 174.8 ± 10 μ m ($n = 11$, A) and 192.2 ± 15 μ m ($n = 12$, B).

ferase (7 μ M, reference 35) by comparison with that of AFC (20 μ M). It is worth to note, at this point, the contrast between the C_{20} isoprenyl cysteine analogue (active) and the C_{20} isoprenol (inactive). This suggests that farnesol and AFC, which have similar vascular properties and share evident structural similarities, actually belong to two different pharmacological families of compounds.

The action of farnesol and AFC was not limited to NE-induced contraction. Both compounds inhibited the response to KCl. They also inhibited NaF-induced contractions. Therefore, it is unlikely that they interact directly with the adrenergic receptor. KCl depolarizes plasma membrane, stimulates voltage-dependent Ca^{2+} channels, and increases intracellular Ca^{2+} levels, thus promoting smooth muscle contraction (36). Although NE-induced contraction is mainly the consequence of the activation of G protein-coupled adrenergic receptors and release of Ca^{2+} from intracellular stores, it is also sustained by a secondary activation of voltage-dependent (L-type) Ca^{2+} channels (37–39). Thus, the inhibitory action of the C_{15} isoprenoids on both receptor- and voltage-mediated contractions is compatible with a direct inhibition of Ca^{2+} channel activity. However, one cannot rule out an effect on G proteins or G protein-dependent pathways since voltage-dependent Ca^{2+} channels, including those found in vascular smooth muscle, are subjected to direct regulation by G proteins (39–44). The results of our experiments with Ach and NaF would actually support this hypothesis since Ach-dependent relaxation results from activation of G protein-coupled muscarinic receptors on the endothelial cells (45), and NaF is a potent activator of all known heterotrimeric G proteins when complexed with aluminum (19, 20, 22, 23). Therefore, it is tempting to propose that farnesol and AFC act primarily on vascular G proteins, with secondary inhibition of G protein-dependent events including Ca^{2+} channel activation.

An effect of AFC and farnesol on either G proteins, Ca^{2+} channels, or both would necessarily be associated with decreased intracellular Ca^{2+} . Indeed, studies performed with platelets reported that AFC decreases intracellular Ca^{2+} con-

centration (46). Such a decrease, if it also occurs in the arterial wall, would then explain the inhibitory effect of AFC on vasoconstriction as well as on Ach-induced relaxation since the activity of endothelial nitric oxide synthase is Ca^{2+} dependent. A similar effect of farnesol on intracellular Ca^{2+} could be postulated. Altogether, our data and those from others suggest a potential action of farnesol and AFC on G protein-dependent signaling pathways controlling Ca^{2+} homeostasis in arteries and call for further exploration of the molecular mechanism of action of each compound.

Unlike large arteries, small arteries (≤ 200 μ m diameter) contribute significantly to the maintenance of peripheral vascular resistance and to arterial pressure control (47). Therefore, we felt it important to determine if farnesyl analogues also affected vasoconstriction in resistance vessels. The results of these experiments, conducted with human resistance arteries, indicate that the farnesyl-dependent regulation of vascular reactivity is active in humans. Furthermore, they revealed a vasodilatory action of both AFC and farnesol on resting arteries. These observations are important in the context of the continuous demand for new pharmacological approaches in cardiovascular research, especially that addressing the control of vascular tone, and suggest that drugs presenting a farnesyl motif might be efficient in the control of arterial pressure in humans.

In conclusion, we have demonstrated that nonsterol, mevalonate-derived compounds containing the farnesyl motif are potent antagonists of vasoconstriction in rat and human vessels. The study provides new directions for evaluating the relationship between blood pressure and cholesterol metabolism as it suggests that intracellular farnesyl derivatives including farnesylated proteins are critical to the maintenance of vascular tone. Because the action of the farnesyl analogues was rapid, specific, and reversible, we propose that drugs containing the farnesyl motif could be developed for use in regulating human arterial pressure. Finally, the study suggests that disorders of the mevalonate pathway leading to decreased farnesyl availability may alter vascular tone and increase systemic blood pressure.

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