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α_1-Adrenoreceptor stimulation causes vascular smooth muscle cell hypertrophy: a possible role for isoprenoid intermediates

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Abstract

We investigated whether contraction-induced agonists such as α_1-adrenoceptor agonists are important regulators of smooth muscle cell hypertrophy by examining the effects of one potent agonists, phenylephrine, on the hypertrophy. Under the experimental conditions used, we found that phenylephrine was potent in inducing α_1-adrenoreceptor-dependent hypertrophy of vascular smooth muscle cells as defined by increased incorporation of [^{14}C]leucine in a dose-dependent fashion. Further, we assessed the effect of lovastatin, an 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, on hypertrophy of cultured vascular smooth muscle cells as defined by the increased incorporation of [^{14}C]leucine caused by phenylephrine. Lovastatin (5–15 μM) caused a significant dose-dependent reduction in [^{14}C]leucine incorporation which was completely prevented in the presence of exogenous mevalonate (100 μM). Exogenous low density lipoprotein (100 μg/ml) and cholesterol (15 μg/ml) did not prevent lovastatin inhibition of [^{14}C]leucine incorporation. In contrast, the isoprenoid farnesol largely prevented inhibition of [^{14}C]leucine incorporation by the lovastatin. We conclude that mevalonate metabolites are essential for phenylephrine-induced smooth muscle cell hypertrophy, possibly through the production of the isoprenoid farnesol. © 1998 Elsevier Science B.V.

Keywords: α_1-Adrenoreceptor; Hypertrophy; Smooth muscle cell; Lovastatin; Farnesol

1. Introduction

There is considerable interest in the cellular mechanisms involved in the control of vascular smooth muscle growth and their role in the etiology of hypertension and atherosclerosis. It has been demonstrated that aortic smooth muscle cells are capable of two distinct cellular growth responses in vivo, depending on the mode of growth stimulation (Geisterfer et al., 1988; Noveral and Grunstein, 1994). Coarctation models and experimental injury models of atherosclerosis are characterized by extensive proliferation and intimal migration of aortic smooth muscle cells (Owens and Reidy, 1985). In contrast, in Goldblatt rat (Owens and Schwartz, 1983) and spontaneously hypertensive rats (Olivetti et al., 1982), the increase in aortic smooth muscle mass can be accounted for primarily by enlargement of existing cells, or cellular hypertrophy, rather than cellular proliferation.

There is indirect evidence that smooth muscle cell hypertrophy may represent a response to increased pressure. Furthermore, pharmacological lowering of blood pressure can prevent development of smooth muscle cells hypertrophy and can reverse cellular hypertrophy that already has occurred in spontaneously hypertensive rats (Owens, 1987; Yamori et al., 1980). However, these data must be interpreted with caution because there is no direct evidence that these effects on growth are due to lowering of blood pressure as opposed to some other direct or indirect effect of the drugs on cellular DNA and protein synthesis. In fact, drug intervention studies in those laboratories have demonstrated dissociation of blood pressure changes and smooth muscle cell hypertrophy in spontaneously hypertensive rats treated with different antihypertensive drugs (Owens, 1987; Yamori et al., 1980). Results suggested that the medial degree of smooth muscle cell hypertrophy was not simply a response to increased blood pressure but that it implicated a direct role for specific contractile agonists in control of smooth muscle cell growth. Therefore, the hypothesis that contractile agonists, for example α_1-adrenoreceptor agonists such as phenyl-

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ephrine, might play some direct role in mediation of smooth muscle cell hypertrophy is of interest.

Lovastatin is used in the treatment of hypercholesterolaemia and has been shown to prevent restenosis in a hypercholesterolaemic rabbit model of restenosis (Gellman et al., 1991). Lovastatin competitively inhibits 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (Krukemyer and Talbert, 1987), that is the rate-limiting enzyme in cholesterol biosynthesis. Mevalonate, which is the product of HMG-CoA reductase, is reported to be essential for DNA synthesis (Fairbanks et al., 1984) and is the precursor of a number of non-sterol isoprenoid compounds, including farnesol (Goldstein and Brown, 1990). Covalent bonding of farnesol to certain $P21^{ras}$ proteins and nuclear envelope proteins has recently been shown to be essential for their growth-regulating properties (Casey et al., 1989). In addition, it has recently been reported that lovastatin inhibits proliferation in cultured vascular smooth muscle cells (Munro et al., 1994).

We have examined the possible role of one contractile α_1-adrenoreceptor agonist, phenylephrine, in regulation of vascular smooth muscle cell hypertrophy as defined by increased incorporation of [^{14}C]leucine and the inhibitory properties of lovastatin on phenylephrine-induced increase of [^{14}C]leucine incorporation. Furthermore, we have determined whether this inhibitory activity was due to depletion of cellular mevalonate, cholesterol or the non-sterol isoprenoids.

2. Materials and methods

2.1. Materials

DL-Mevalonic acid lactone, geraniol, farnesol, squalene, cholesterol and α-hydroxyfarnesyl phosphonoic acid were obtained from Sigma. Cell culture reagents were from Gibco. [^{14}C]leucine was from Amersham. Lovastatin was generously provided by Merck, Sharp and Dohme.

2.2. Cell culture

Vascular smooth muscle cells were obtained from thoracic aorta of the rat by the method previously described (Nishio and Watanabe, 1997). The cells (1×10^5) were seeded into 35-mm diameter dishes and maintained in 2 ml of Dulbeco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum at 37°C in a humidified atmosphere of 5% CO_2/95% air. The cells were used between the third and sixth passage. The phenotypic characteristics was examined based on the proliferative response to fetal bovine serum or platelet-derived growth factor-BB (data not shown). There was no difference in proliferative response between the third and sixth passage. Cells were grown to confluence, at which time they were rendered quiescent by the DMEM medium containing 0.5% (v/v) fetal bovine serum and maintained for 72 h before experimentation. The number of viable cells was determined in duplicate by hematocytometry with Trypan blue exclusion. At confluence, the number of viable cells did not vary from dish to dish (data not shown). Viable cells is beyond 95% inspite of various treatment. The cell number is nearly equal to viable cells in dish. Therefore, [^{14}C]leucine incorporation in a dish is nearly equal to normalization of the data by the exact number of the viable cells in a dish.

2.3. Preparation of lovastatin

To convert the inactive lactone form of lovastatin to the active form, the drug was dissolved in ethanol, heated at 50°C in 0.1 M NaOH, neutralized with HCl, and stored unfiltered at −20°C as a 4 mg/ml stock (Nishio et al., 1996b).

2.4. Lipoprotein preparation

Low density lipoprotein was prepared from human plasma from fasted normolipidemic volunteers. Low density lipoprotein was prepared by discontinuous density gradient ultracentrifugation as described previously (Nishio et al., 1996a).

2.5. Measurement of rat vascular smooth muscle cell hypertrophy and cell number

Serum-starved rat vascular smooth muscle cells were treated for 24 h with DMEM containing phenylephrine and 0.5% fetal bovine serum in the presence of 0.5 μCi [^{14}C]leucine per 16-mm well. Thereafter, the medium was aspirated, cells washed three times with cold phosphate-buffered saline, pH 7.0, then washed once with 10% trichloroacetic acid, and incubated at 4°C for 30 min in 10% trichloroacetic acid. Acid-insoluble material was hydrolyzed by the addition of 0.25 M NaOH, and label incorporation was determined by liquid scintillation spectroscopy (Schmidt et al., 1982). For cell number determination, no radioligands were added. The cells were gently trypsinized and counted in a Coulter counter.

2.6. Statistics

Values are expressed as the arithmetic mean ± S.D. Statistical analysis of the data was performed by the one-way analysis of variance (ANOVA), followed by the Scheffe's test when F ratios were significant ($P < 0.05$).

3. Results

3.1. Effect of phenylephrine on [^{14}C]leucine incorporation and cell number in quiescent confluent vascular smooth muscle cells

To study the effects of phenylephrine on vascular smooth muscle cells hypertrophy in culture, we measured

alterations in cell number and the incorporation of [^{14}C]leucine in quiescent confluent vascular smooth muscle cells. Phenylephrine caused a dose-dependent increase in [^{14}C]leucine incorporation in the cells (Fig. 1). No significant changes in cell number were observed under the experimental conditions.

To determine the specificity of α_1-adrenoreceptor in 10 μM phenylephrine-stimulated [^{14}C]leucine incorporation, vascular smooth muscle cells were stimulated with phenylephrine in the presence of the α_1-adrenoreceptor-selective antagonist, prazosin or the α_2-adrenoreceptor-selective antagonist, yohimbine. Fig. 2 shows the effects of the antagonist on [^{14}C]leucine incorporation induced by 10 μM phenylephrine in VSMCs. Prazosin at 1 μM significantly inhibited the phenylephrine-stimulated response. Furthermore, prazosin at 100 μM completely inhibited the phenylephrine-stimulated [^{14}C]leucine incorporation. The IC_{50} value of prazosin was 1.2 ± 0.09 μM in vascular smooth muscle cells. Under the same experimental conditions, yohimbine showed no inhibition in 10 μM phenylephrine-stimulated vascular smooth muscle cell [^{14}C]leucine incorporation even at 100 μM. These results indicate that phenylephrine-induced vascular smooth muscle cell hypertrophy as defined by increased incorporation of [^{14}C]leucine was mediated by the stimulation of the α_1-adrenoreceptor in the cells.

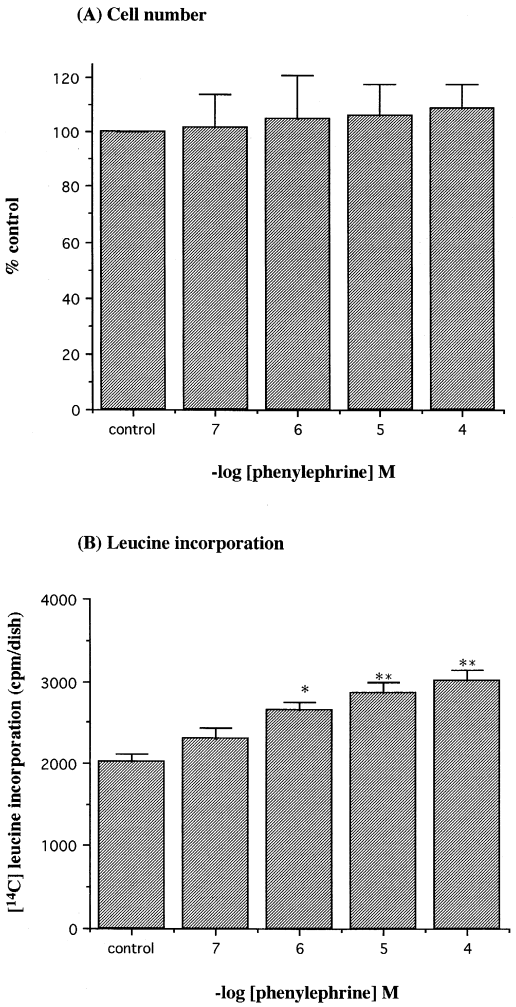

Fig. 1. Effects of phenylephrine on SMC cell number and [^{14}C]leucine incorporation. Cells were growth-arrested in 0.5% fetal bovine serum for 72 h. Thereafter, the cells were treated for 24 h with DMEM containing the various concentrations of phenylephrine and 0.5% fetal bovine serum in the presence of 0.5 μCi [^{14}C]leucine. (A) Cell number was measured as described in Section 2. Values are means ± S.D. of the cell number as a percentage of non-treated cells in three independent experiments in duplicate. (B) [^{14}C]leucine incorporation/dish was measured as described in Section 2. Results are the mean ± S.D. of three independent experiments in duplicate. $* P < 0.05$, $** P < 0.01$ compared with the control.

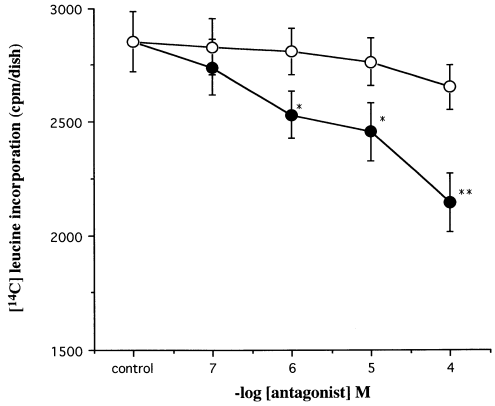

Fig. 2. Effects of prazosin (●) and yohimbine (○) on the phenylephrine (10 μM)-stimulated [^{14}C]leucine incorporation. Growth-arrested vascular smooth muscle cells were treated with phenylephrine (10 μM) and the various antagonists. [^{14}C]leucine incorporation was measured over a 24 h period as described in Section 2. Each point represents the mean ± S.D. of three independent experiments in triplicate. $* P < 0.05$, $** P < 0.01$ compared with the control.

3.2. Effect of lovastatin on the hypertrophy of vascular smooth muscle cells induced by phenylephrine

When lovastatin was added to the culture, a significant dose-dependent decrease in phenylephrine (10 μM)-stimulated [^{14}C]leucine incorporation. At a concentration of 5 μM, [^{14}C]leucine incorporation was reduced significantly as compared with phenylephrine (10 μM)-treated cultures (Fig. 3). Lovastatin alone had no effect on [^{14}C]leucine incorporation as compared with the control, nor was there any microscopic evidence of a cytotoxic effect of lovastatin.

3.3. Effect of mevalonate and its isoprenoid derivatives on the inhibition by lovastatin of phenylephrine-induced [^{14}C]leucine incorporation

To investigate whether lovastatin inhibits phenylephrine-induced [^{14}C]leucine incorporation by the inhibition of cholesterol synthesis or by isoprenoid synthesis, we

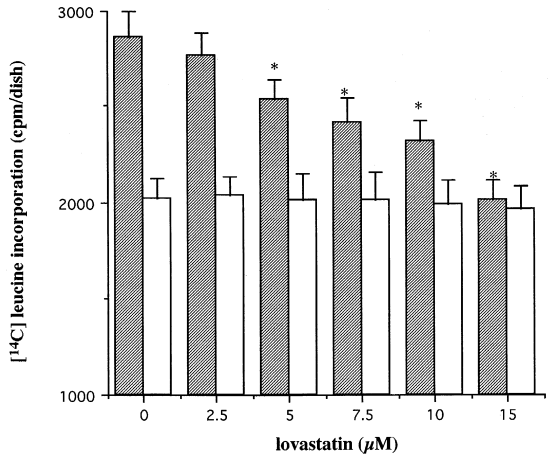

Fig. 3. Effects of lovastatin on phenylephrine (10 μM)-stimulated [^{14}C]leucine incorporation in vascular smooth muscle cells. Growth-arrested vascular smooth muscle cells were incubated with indicated concentrations of lovastatin in the presence (shaded bar) or absence (open bar) of phenylephrine (10 μM). [^{14}C]leucine incorporation was measured over at 24 h period as described in Section 2. The result represents the mean $\pm$ S.D. of three independent experiments in triplicate. $* P < 0.01$ compared with phenylephrine (10 μM) alone.

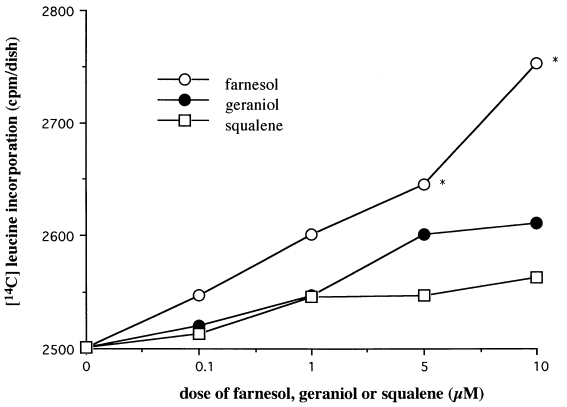

Fig. 5. Effect of mevalonate derivatives on the lovastatin (5 μM) inhibition of phenylephrine (10 μM)-stimulated [^{14}C]leucine incorporation. Growth-arrested vascular smooth muscle cells were incubated with phenylephrine (10 μM) and lovastatin (5 μM) in the presence of mevalonate derivatives. [^{14}C]leucine incorporation was measured over a 24-h period as described in Section 2. Each point represents the mean of three independent experiments performed in triplicate. $* P < 0.05$ compared with phenylephrine (10 μM) and lovastatin (5 μM) in combination.

added cholesterol or mevalonic acid to reverse the lovastatin-induced inhibition. Mevalonic acid significantly prevented the inhibitory effect of lovastatin at 100 μM (Fig. 4). Mevalonic acid alone had no effect on [^{14}C]leucine incorporation. Furthermore, geraniol and farnesol, at the highest but non-toxic, concentrations tested, partially prevented the inhibitory effect of lovastatin. However, squalene caused no remarkable prevention of lovastatin-induced inhibitory effects (Fig. 5). There was not a significant difference between the effect of geraniol and squalene on lovastatin-induced inhibitory effects. These results demonstrated that smooth muscle cells require mevalonate itself or some of its non-sterol products such as farnesol or geraniol for phenylephrine-stimulated [^{14}C]leucine incorporation.

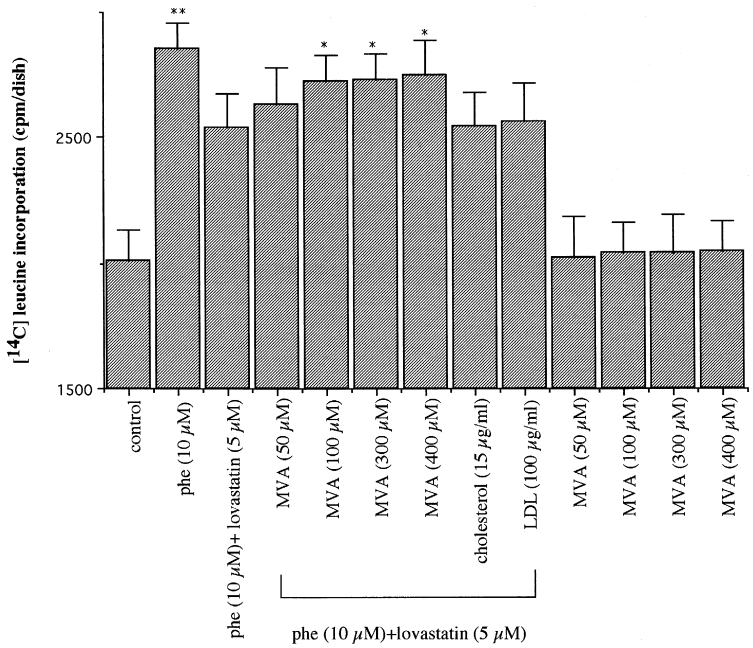

Fig. 4. Effect of mevalonic acid (MVA) and cholesterol on the inhibition of phenylephrine (10 μM)-stimulated [^{14}C]leucine incorporation by lovastatin. Growth-arrested vascular smooth muscle cells were incubated with phenylephrine (10 μM) and lovastatin (5 μM) in the presence of MVA, cholesterol or LDL. [^{14}C]leucine incorporation was measured over a 24 h period as described in Section 2. Each bar represents the mean $\pm$ S.D. of three independent experiments in triplicate. $* P < 0.05$, $** P < 0.01$ compared with phenylephrine (10 μM) and lovastatin (5 μM) in combination.

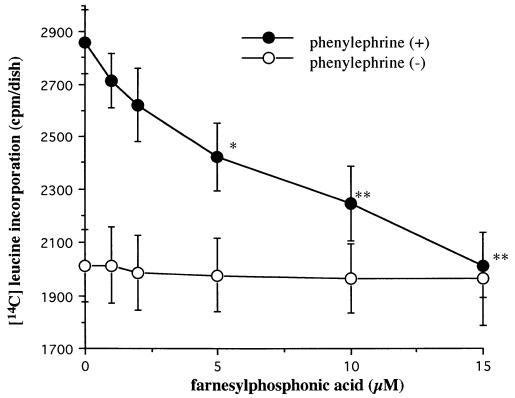

Fig. 6. Effect of farnesol phosphonic acid on the phenylephrine (10 μM)-stimulated [^{14}C]leucine incorporation. Growth-arrested vascular smooth muscle cells were incubated with farnesol phosphonic acid in the presence or absence of phenylephrine (10 μM). [^{14}C]leucine incorporation was measured over a 24-h period as described in Section 2. Each bar represents the mean $\pm$ S.D. of three independent experiments performed in triplicate. $* P < 0.05$, $** P < 0.01$ compared with phenylephrine (10 μM) alone.

3.4. Effect of farnesol transferase inhibitor on the phenylephrine-induced [^{14}C]leucine incorporation

To verify the involvement of farnesol in phenylephrine-induced [^{14}C]leucine incorporation, we investigated whether the farnesol transferase inhibitor, α-hydroxyfarnesyl phosphonic acid, would mimic the effect of lovastatin-induced inhibition of [^{14}C]leucine incorporation. Fig. 6 demonstrated that farnesol transferase inhibitor impaired phenylephrine (10 μM) stimulation of [^{14}C]leucine incorporation in a dose-dependent manner. The IC_{50} was 5 ± 0.3 μM. Farnesol transferase inhibitor alone had no effect on [^{14}C]leucine incorporation as compared with the control, nor was there any microscopic evidence of a cytotoxic effect of farnesol transferase inhibitor. The latter was established by morphology and Trypan blue exclusion, within the concentrations used.

4. Discussion

In the present study, we have explored the hypothesis that α_1-adrenoreceptors are involved in cultured vascular smooth muscle cell hypertrophy as defined by increased incorporation of [^{14}C]leucine. Under the conditions examined, we found that phenylephrine was potent in inducing α_1-adrenoreceptor-dependent [^{14}C]leucine incorporation of smooth muscle cells in a dose-dependent fashion. Furthermore, we investigated the effect of lovastatin on the phenylephrine-induced increase of [^{14}C]leucine incorporation of smooth muscle cell. Lovastatin was found to inhibit the increase of [^{14}C]leucine incorporation of smooth muscle cells caused by phenylephrine (10 μM) with an IC_{50} value of 7.5 μM.

Inhibition of myointimal hyperplasia by lovastatin could occur through a reduction in total serum cholesterol, although large clinical trials have found no correlation between serum cholesterol and restenosis (Leimgruber et al., 1986; Guiteras et al., 1987). In fact, the addition of low density lipoprotein and cholesterol did not reverse the inhibitory effect of lovastatin. A more likely mechanism is inhibition of mevalonate, the direct product of HMG-CoA reductase synthesis, and its derivatives. Mevalonate has been shown to be essential for proliferation in a number of cell types (Siperstein, 1984), including vascular smooth muscle cells (Munro et al., 1994). We have found that supplementation with mevalonate restores the inhibition by lovastatin on the phenylephrine-induced increase of [^{14}C]leucine incorporation. Furthermore, mevalonate at this concentration did not appear to induce increase of [^{14}C]leucine incorporation in vascular smooth muscle cells, as there was no marked incorporation of [^{14}C]leucine with mevalonate alone. Reversal of the inhibition of [^{14}C]leucine incorporation was not significantly different for 100 μM or for 400 μM mevalonate, suggesting that a lower concentration of mevalonate was sufficient (Fig. 4).

The current results suggest that non-sterol metabolites of mevalonate, possibly prenylated proteins, are involved in diverse cellular functions. A number of non-sterol isoprenoid compounds are synthesized from mevalonate; dolichol which is essential for N-linked glycosylation (Dan et al., 1996), a critical step in glycoprotein synthesis; ubiquinone, an important component of the respiratory chain (Robinson and Lemire, 1996); isopentenyl adenine, incorporated into some t-RNAs (Houssier and Grosjean, 1985); and farnesol, covalently bonded to Ras and lamin B proteins (Bruscalupi et al., 1994).

One isoprenoid that may be critical for cellular function is farnesol. Some intracellular proteins bind farnesol covalently, including Ras proteins and other Ras-related, low molecular mass, GTP-binding proteins (Mumby et al., 1990). Farnesylated $P21^{ras}$ proteins attach to the inner surface of the cell membrane, and may serve as signaling mechanisms for cellular function (Santos and Nebreda, 1989). In the present study, therefore, it is possible that lovastatin inhibits smooth muscle cell [^{14}C]leucine incorporation, at least in part, by blocking the production of farnesol and farnesylated proteins. Our results of the experiment using exogenous farnesol (Fig. 5) support this possibility. Also, our findings that farnesyltransferase inhibitor impaired the phenylephrine-induced [^{14}C]leucine incorporation in a dose-dependent manner (Fig. 6), support this possibility.

It is difficult to compare the effective concentrations of lovastatin in the present experiments with plasma levels of lovastatin in vivo studies. Lovastatin is administered as an inactive lactone form and undergoes hydrolysis to the β-hydroacid or open acid form. In addition, lovastatin acid undergoes extensive hepatic metabolism to produce at least nine other metabolites (Duggan et al., 1989), some of

which retain inhibitory activity against HMG-CoA reductase.

In summary, results of the present study demonstrate that phenylephrine, α_1-adrenoreceptor agonist, is a potent hypertrophic agent for cultured rat vascular smooth muscle cells. The study demonstrated the involvement of mevalonate and its derivatives in smooth muscle cell hypertrophy. Further studies are required to determine the cellular mechanisms responsible for phenylephrine-induced hypertrophy and to define the target protein for farnesylation which is involved in hypertrophy.

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References

Bruscalupi, G., Castellano, F., Sacco, S., Trentalance, A., 1994. Prenylated proteins in regenerating rat liver. Biochem. Biophys. Res. Commun. 200, 1713–1720.

Casey, P.J., Solski, P.A., Der, C.J., Buss, J.E., 1989. $P21^{ras}$ is modified by a farnesyl isoprenoid. Proc. Natl. Acad. Sci. USA 86, 8323–8327.

Dan, N., Middleton, R.B., Lehrman, M.A., 1996. Hamster UDP-*N*-acetylglucosamine: dolichol-*P* *N*-acetylglucosamine-1-*P*-transferase has multiple transmembrane spans and a critical cytosolic loop. J. Biol. Chem. 271, 30717–30724.

Duggan, D.E., Chen, I.W., Bayne, W.F., Halpin, R.A., Duncan, C.A., Schwattz, M.S., Stubbs, R.J., Vickers, S., 1989. The physiological disposition of lovastatin. Drug Metab. Dispos. 17, 166–173.

Fairbanks, K.P., Witte, L.D., Goodman, D.S., 1984. Relationship between mevalonate and mitogenesis in human fibroblasts stimulated with platelet-derived growth factor. J. Biol. Chem. 259, 1546–1551.

Geisterfer, A.A., Peach, M.J., Owens, G.K., 1988. Angiotensin II induces hypertrophy, not hyperplasia, of cultured rat aortic smooth muscle cells. Circ. Res. 62, 749–756.

Gellman, J., Ezekowitz, M.D., Sarembock, I.J., Azrin, M.A., Nochomowitz, L.E., Lerner, E., Haudenschild, C.C., 1991. Effect of lovastatin on intimal hyperplasia after balloon angioplasty: a study in an atherosclerotic hypercholesterolemic rabbit. J. Am. Coll. Cardiol. 17, 251–259.

Goldstein, J.L., Brown, M.S., 1990. Regulation of the mevalonate pathway. Nature 343, 425–430.

Guiteras, V.P., Bourassa, M.G., David, P.R., Bonan, R., Crepeau, J., Dyrda, I., Lesperance, J., 1987. Restenosis after successful percutaneous transluminal coronary angioplasty: the Montral Heart Institute experience. Am. J. Cardiol. 60, 50B–55B.

Houssier, C., Grosjean, H., 1985. Temperature jump relaxation studies on the interactions between transfer RNAs with complementary anticodons. The effect of modified bases adjacent to the anticodon triplet. J. Biomol. Struct. Dyn. 3, 387–408.

Krukemyer, J.J., Talbert, R.L., 1987. Lovastatin: a new cholesterol-lowering agent. Pharmacotherapy 7, 198–210.

Leimgruber, P.P., Roubin, G.S., Hollman, J., Cotsonis, G.A., Meier, B., Douglas, J.S., King, S.J., Gruentzig, A.R., 1986. Restenosis after successful coronary angioplasty in patient with single-vessel disease. Circulation 73, 710–717.

Mumby, S.M., Casy, P.J., Gilman, A.G., Gutowski, S., Sternweis, P.C., 1990. G protein gamma subunits contain a 20-carbon isoprenoid. Proc. Natl. Acad. Sci. USA 87, 5873–5877.

Munro, E., Patel, M., Chan, P., Betterige, L., Clunn, G., Gallagher, K., Hughes, A., Schachter, M., Wolfe, J., Sever, P., 1994. Inhibition of human vascular smooth muscle cell proliferation by lovastatin: the role of isoprenoid intermediates of cholesterol synthesis. Eur. J. Clin. Invest. 24, 766–772.

Nishio, E., Arimura, S., Watanabe, Y., 1996a. Oxidized LDL induces apoptosis in cultured smooth muscle cells: a possible role for 7-ketocholesterol. Biochem. Biophys. Res. Commun. 223, 413–418.

Nishio, E., Tomiyama, K., Nakata, H., Watanabe, Y., 1996b. 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitor impairs cell differentiation in cultured adipogenic cells (3T3-L1). Eur. J. Parmacol. 301, 203–206.

Nishio, E., Watanabe, Y., 1997. The involvement of reactive oxygen species and arachidonic acid in α_1-adrenoreceptor-induced smooth muscle cell proliferation and migration. Brit. J. Pharmacol. 121, 665–670.

Noveral, J.P., Grunstein, M.M., 1994. Adrenergic receptor-mediated regulation of cultured rabbit airway smooth muscle cell proliferation. Am. J. Physiol. 267, L291–299.

Olivetti, G., Melissari, M., Marchetti, G., Anversa, P., 1982. Quantitative structural changes of the rat thoracic aorta in early spontaneous hypertension. Tissue composition, and hypertrophy and hyperplasia of smooth muscle cells. Circ. Res. 51, 19–26.

Owens, G.K., 1987. Influence of blood pressure on development of aortic medial smooth muscle hypertrophy in spontaneously hypertensive rats. Hypertension 9, 178–187.

Owens, G.K., Schwartz, S.M., 1983. Vascular smooth muscle cell hypertrophy and hyperploidy in the Goldblatt hypertensive rat. Circ. Res. 53, 491–501.

Owens, G.K., Reidy, M.A., 1985. Hyperplastic growth response of vascular smooth muscle cells following induction of acute hypertension in rats by aortic coarctation. Circ. Res. 57, 695–705.

Robinson, K.M., Lemire, B.D., 1996. Covalent attachment of FAD to the yeast succinate dehydrogenase flavoprotein requires import into mitochondria, presequence removal, and folding. J. Biol. Chem. 271, 4055–4060.

Santos, E., Nebreda, A.R., 1989. Structural and functional properties of ras proteins. FASEB J. 3, 2151–2163.

Schmidt, R.A., Glomset, J.A., Wight, T.N., Habenicht, A.J.R., Ross, R., 1982. A study of the influence of mevalonic acid and its metabolites on the morphology of Swiss 3T3 cells. J. Cell Biol. 95, 144–153.

Siperstein, M.D., 1984. Role of cholesterogenesis and isoprenoid synthesis in DNA replication and cell growth. J. Lipid Res. 25, 1462–1468.

Yamori, Y., Tarazi, R.C., Ooshima, A., 1980. Effect of beta-receptor-blocking agents on cardiovascular structural changes in spontaneous and noradrenaline-induced hypertension in rats. Clin. Sci. 59, 457s–460s.