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Mevalonate availability and cardiovascular functions

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ABSTRACT **Data delineating the relationship between disorders of cholesterol metabolism and elevated blood pressure (BP) do not exist. We postulated that mevalonate, the metabolic precursor of endogenous cholesterol and the direct product of 3-hydroxy-3-methylglutaryl-CoA reductase, was a contributing factor for the maintenance of vascular tone and systemic BP. We conducted *in vivo*, *ex vivo*, and *in vitro* experiments in normotensive and hypertensive rats, where exogenous mevalonate and lovastatin, a competitive inhibitor of 3-hydroxy-3-methylglutaryl-CoA reductase, were used, respectively, to increase or limit mevalonate availability. Mevalonate decreased BP in the whole animal without significant change in plasma cholesterol. Incubation of aortas with mevalonate attenuated their reactivity to norepinephrine and increased their response to endothelium-dependent and -independent relaxing factors. Lovastatin, in contrast, had the opposite effect *in vivo* and *in vitro*: it increased BP, enhanced vascular response to norepinephrine, and impaired endothelium-dependent and -independent relaxations. Neither agent modified cholesterol vascular content. Alteration of vascular reactivity was also observed in resistance vessels from animals pretreated with lovastatin. Our findings suggest that mevalonate availability is an unrecognized metabolic contributor to vascular tone and BP. They imply that (*i*) metabolites of the mevalonate pathway other than cholesterol could potentially control vascular functions and cardiovascular hemodynamics, (*ii*) elevated arterial pressure could be in part the consequence of primary disorders of this pathway, and (*iii*) pharmacological inhibition of mevalonate production as a means to lower plasma cholesterol may have an adverse impact on other cardiovascular risk factors, such as BP.**

Essential hypertension is frequently associated with atherosclerosis (1, 2). The adverse influence of hypercholesterolemia and atherosclerosis as pathogenetic factors has been intensely investigated. Cholesterol enrichment of the plasma membrane of vascular myocytes adversely modifies cellular calcium kinetics and the cell response to various hormones and mediators (3–6), whereas that of rabbit arteries increases their sensitivity to adrenergic stimulation (7). By altering vascular reactivity, hypercholesterolemia and atherosclerosis could potentially contribute to blood pressure (BP) elevation. However, there has been no clinical or experimental demonstration that elevation of circulating cholesterol necessarily increases systemic BP. Also, a recent report showed paradoxically that cholesterol enrichment of the smooth muscle cell membrane, if it does increase reactivity to vasoconstrictors, can also increase the sensitivity to vasodilators (8). Finally, reduction in plasma cholesterol, either by dietary restriction or by pharmacological treatment has not been reported to decrease BP significantly. Thus, there is no direct cause–effect relationship between exogenous cholesterol (circulating or accumulating in cell membrane) and BP control.

Cell cholesterol homeostasis is dependent not only on exogenous availability of the sterol but also on endogenous synthesis. Intracellular cholesterol synthesis is part of a very tightly regulated system (the mevalonate pathway) that satisfies the cell requirement not only for cholesterol but also for various nonsterol (isoprenoid) products such as dolichol, ubiquinone, and prenylated proteins. The participation of these nonsterol metabolites in vascular physiology and BP regulation is essentially unknown. However, indications that they might play a major role can be inferred from published work (9–11). In the mevalonate pathway, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase is a critical rate-limiting step (12), ensuring adequate supply of mevalonate for sterol and nonsterol synthesis. Modulation of mevalonate production and cellular availability can be achieved with competitive inhibitors of the enzyme, such as lovastatin. The drug, which has been utilized extensively to study the regulation of cholesterol synthesis, has recently been used to elucidate the existence of covalent isoprenylation of cellular proteins and the functional role of protein prenylation. In *in vitro* studies exploring the functional impact of blocking HMG-CoA reductase activity with lovastatin, the specificity of action of lovastatin has been tested by the ability of mevalonate to restore normal cell function.

Considering the absence of a direct relationship between plasma cholesterol and BP and the potential role of endogenous cholesterol and nonsterol isoprenoids in vascular physiology, we postulated that mevalonate availability directly influences vascular functions and, ultimately, BP. The hypothesis was tested *in vivo*, *ex vivo*, and *in vitro* using the rat as a standard laboratory model of arterial pressure regulation.

METHODS

***In Vivo* Experiments.** Normotensive Wistar Kyoto (WKY) and spontaneously hypertensive (SHR) rats were chosen. In a first experiment, 12 WKY and 12 SHR (14- to 16-week-old males, Charles River Breeding Laboratories) rats were allocated either to the mevalonate group (M group) or to the control group (C group). Systolic BP was indirectly measured (tail-cuff method, Narco system) from day 0 to day 21. The gavage was started at day 1. M rats received 250 mg of mevalonate per day (DL-mevalonic acid lactone, Sigma M 9627). C rats received the vehicle (water) only. At day 11, the M group was switched to vehicle and the C group was switched to mevalonate. Plasma levels of total cholesterol were determined at days 11 and 22 after a 16- to 18-hr fast and 24 hr after the last gavage. In a second experiment, rats of each strain (10- to 12-week-old males, $n = 12$ per strain) were allocated either to the lovastatin group (L group) or to the

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Abbreviations: BP, blood pressure; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; NE, norepinephrine; SNP, sodium nitroprusside; WKY, normotensive Wistar Kyoto; SHR, spontaneously hypertensive; FC, free cholesterol; PL, phospholipid(s).

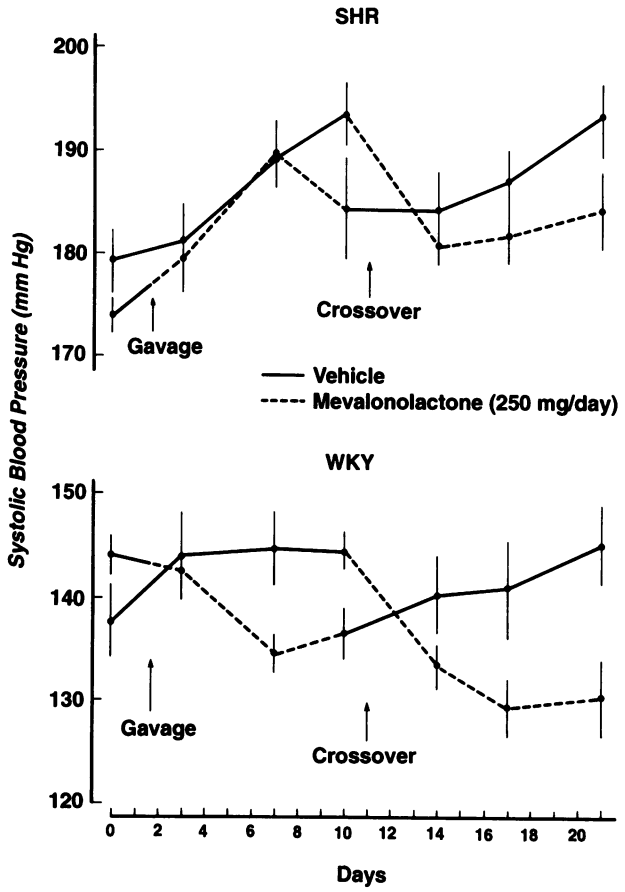

FIG. 1. Systolic BP of WKY and SHR rats given daily administration of either mevalonolactone or vehicle. $n = 6$ animals per group.

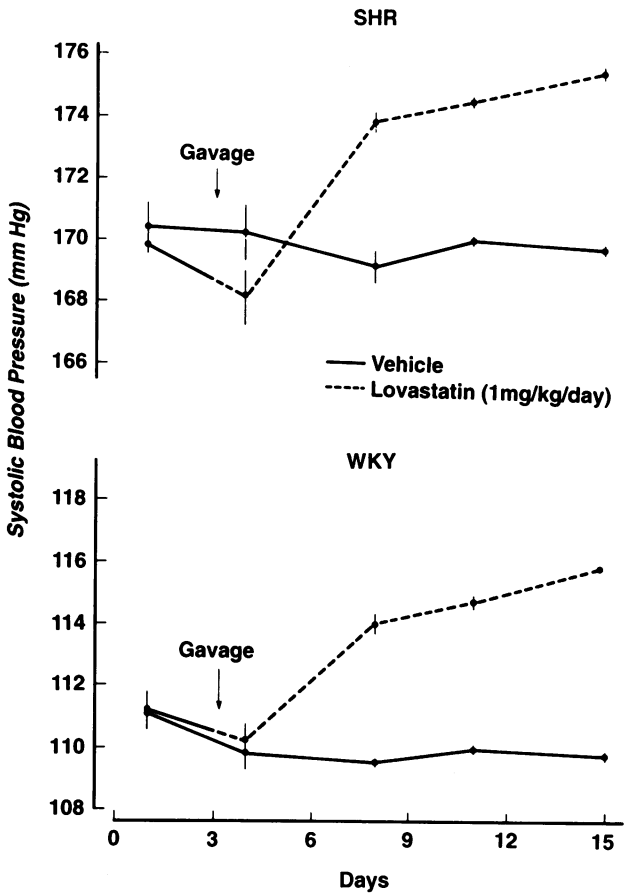

FIG. 2. Systolic BP of WKY and SHR rats given daily administration of either lovastatin or vehicle. $n = 6$ animals per group.

control group (C group). L rats received 1 mg of lovastatin (a gift from Merck Sharp & Dohme) per kg (body weight) per day, suspended in an aqueous solution of dimethyl sulfoxide [10% (vol/vol) in water]. C rats received the vehicle only. The gavage was started at day 3 for 13 days. BP was measured repeatedly. Plasma cholesterol levels were determined on day 16.

***Ex Vivo* Experiments.** Thirty normotensive rats (14- to 16-week-old male WKY) were allocated to either the L group (1 mg/kg per day), the M group (250 mg/day), or the C group ($n = 10$ for each group) after a baseline period of 1 wk. The drugs or vehicle [10% (vol/vol) dimethyl sulfoxide in water] were administered by gavage for 13 days. BP was measured repeatedly throughout the experimental period. On day 14, after sacrifice, the mesenteric artery bed was excised and rinsed with saline. Resistance vessels (from the second branch of the mesenteric artery) were mounted in a myograph chamber and their reactivity was tested as described (13). The response (vasoconstriction) to norepinephrine (NE) was first examined. Then, the reactivity (relaxation) to acetylcholine, a stimulant of endothelium-derived relaxing factor (14), and to sodium nitroprusside (SNP), a direct activator of the smooth muscle cell soluble guanylate cyclase (15), was assayed after precontraction with NE.

***In Vitro* Experiments.** In this set of experiments, vessels from nontreated (normotensive) animals were exposed directly to lovastatin or mevalonate. After ether anesthesia, the thoracic aorta from either Wistar (12- to 16-week-old males, $n = 8$) or WKY (12- to 16-week-old males, $n = 6$) rats was dissected out, and fat and connective tissue were removed. Aortic segments were incubated in Dulbecco's modified Eagle medium (DMEM) supplemented with antibiotics at 37°C in a 5% CO_2 atmosphere for 24, 48, and 72 hr (Wistar) or for 48 hr only (WKY). Lovastatin (5 μg/ml), mevalonolactone (10 mM), lovastatin and mevalonolactone (5 μg/ml and 10 mM, respectively), or vehicle [0.1% (vol/vol) dimethyl sulfoxide] was present during the whole incubation. Vascular reactivity was determined in a muscle bath apparatus as described (16) in the absence of extracellular drug. Finally, the impact of the various drugs on aortic free cholesterol (FC) and phospholipids (PL) was estimated in parallel aorta preparations obtained from Wistar rats (12- to 16-week-old males, $n = 9$). Aortic lipids were extracted according to Folch *et al.* (17). FC (free cholesterol C kit, Wako Biochemicals, Osaka) and PL phosphorus (18) were determined.

Statistical Analysis. All results are expressed as mean ± SEM. Two-tailed Student's t tests (paired or unpaired) and analysis of variance (ANOVA) were used when appropriate, with a significance level of $P < 0.05$.

RESULTS

***In Vivo*.** As shown in Fig. 1, mevalonate significantly decreased BP ($P < 0.004$, ANOVA) in both strains (experiment 1). SHR rats manifested greater spontaneous variation of BP than WKY rats, thus obscuring the effect of mevalonate in the hypertensive strain. However, the response to the drug was statistically similar for both strains ($P = 0.14$, nonsignificant for the interaction strain per drug). Plasma cholesterol levels were lower in SHR than in WKY rats ($P < 0.001$) but were not affected by the drug treatment. Levels (mM) for the C group were (day 11 vs. day 22) 1.91 ± 0.04 vs. 1.91 ± 0.06 (WKY) and 1.05 ± 0.04 vs. 1.19 ± 0.03 (SHR). Levels for the M group were 1.89 ± 0.04 vs. 1.93 ± 0.05 (WKY) and 1.04 ± 0.03 vs. 1.06 ± 0.04 (SHR). Lovastatin

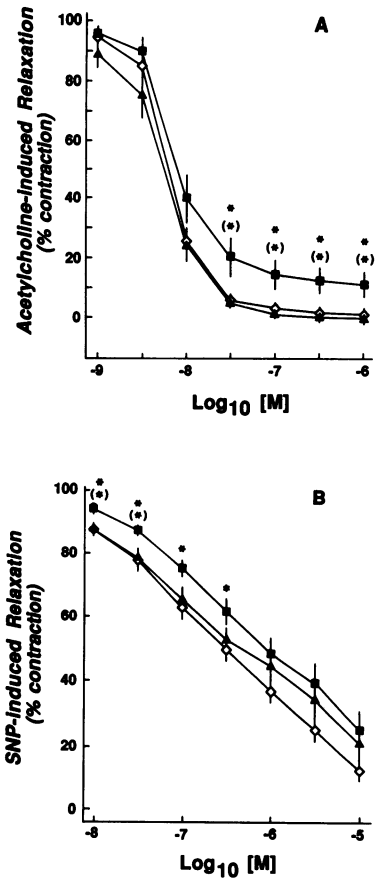

FIG. 3. Reactivity to acetylcholine (*A*) and SNP (*B*) of resistance vessels (mesenteric artery) isolated from WKY rats treated for 13 days with lovastatin (1 mg/kg, ■), mevalonolactone (250 mg/day, ▲), or vehicle (◇). * and (*) indicate a significant difference with C and M animals, respectively. Each group contains 9 (M and C) or 10 (L) animals.

significantly raised BP ($P < 0.001$) in both strains (Fig. 2, experiment 2). The effect on BP was not observed at day 4, (24 hr of treatment), but progressive increase was detected at days 8, 11, and 15. The plasma cholesterol levels of lovastatin-treated animals were not significantly different from the strain-specific controls: 2.05 ± 0.06 vs. 2.00 ± 0.04 (WKY) and 1.22 ± 0.03 vs. 1.09 ± 0.04 (SHR). Finally, neither vehicle nor mevalonate nor lovastatin affected body weight, food intake, or water intake (data not shown).

Ex Vivo. The effect of lovastatin (increase, $P < 0.007$, ANOVA) and mevalonate (decrease, $P < 0.001$) on BP was confirmed in this set of rats (data not shown). The reactivity of the resistance vessels from lovastatin-treated rats compared to control or mevalonate-treated animals was significantly modified. The maximum response (mN/mm) to NE increased (4.98 ± 0.24 vs. 4.22 ± 0.20 or 4.13 ± 0.20, for C or M rats respectively, $P < 0.03$), and endothelium-dependent (acetylcholine) and -independent (SNP) relaxations decreased (Fig. 3). The vascular reactivity of M rats was not different from that of the control animals.

In Vitro. The experiments conducted with aortic rings from Wistar rats detailed the action of the drugs. The maximum contraction to NE was significantly affected by lovastatin or mevalonate (Fig. 4). The effect was time-dependent and maximum after 72 hr of incubation: maximum contraction was increased in lovastatin-treated segments and decreased in mevalonate-treated ones compared to control. The vessels incubated with both drugs had an intermediate response. Hormone sensitivity as measured by the shift of the dose–response curve was also affected. The effect of both compounds was time-dependent, reaching maximum at 48 hr for lovastatin and 72 hr for mevalonate (not shown). The direction of the changes is best illustrated in the 48-hr curve (Fig. 5): sensitivity increased with lovastatin, decreased with mevalonate, and did not change with a mixture of both agents. The response to acetylcholine was affected by the treatment with lovastatin or mevalonate (Fig. 6). After 24 hr of incubation (Fig. 6*A*), mevalonate dramatically increased the sensitivity of the vessel to the neuromediator and increased its maximum relaxation ($P < 0.005$). The effect of mevalonate was evident even in the presence of lovastatin. Lovastatin alone decreased acetylcholine-induced relaxation. The effect

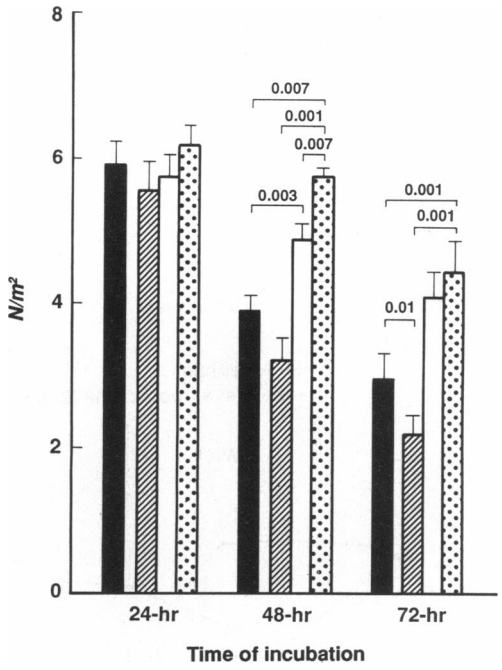

FIG. 4. Maximum contraction to NE of aorta rings (Wistar rats, $n = 8$) incubated for 24, 48, and 72 hr in DMEM containing mevalonate (10 mM, ▨), lovastatin (5 μg/ml, ▩), mevalonate plus lovastatin (□), or vehicle (■). $P < 0.05$ denotes statistical difference.

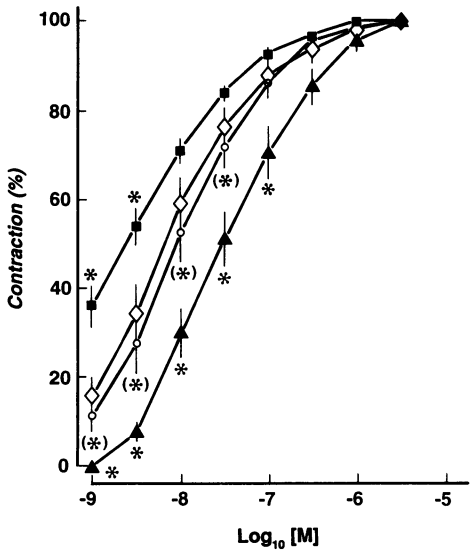

FIG. 5. Sensitivity to NE of aorta rings (Wistar, $n = 8$) incubated for 48 hr in DMEM containing mevalonate (10 mM, ▲), lovastatin (5 μg/ml, ■), mevalonate plus lovastatin (○), or vehicle (◇). * and (*) denote statistical difference with vehicle- and lovastatin-treated preparations, respectively.

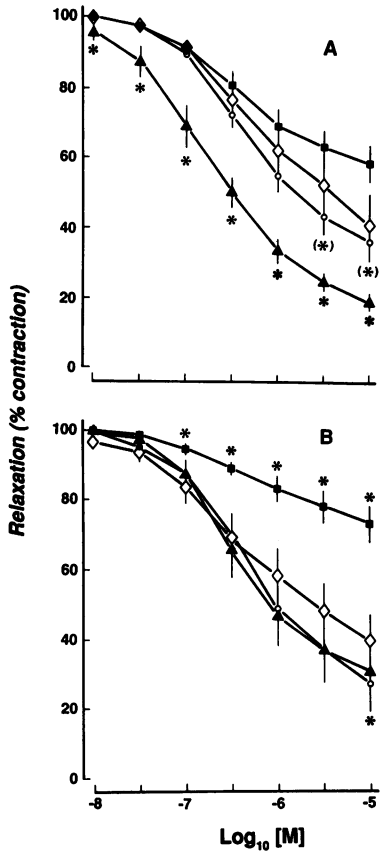

FIG. 6. Reactivity to acetylcholine of aorta rings (Wistar rats, n = 8) incubated for 24 (*A*) and 48 (*B*) hr in DMEM containing mevalonate (10 mM, ▲), lovastatin (5 μg/ml, ■), mevalonate plus lovastatin (○), or vehicle (◇). * and (*) denote statistical difference with vehicle- and lovastatin-treated preparations, respectively.

reached maximum after 48 hr (Fig. 6*B*). Lovastatin significantly decreased the sensitivity and the maximum response to SNP, whereas mevalonate had the opposite effect (Fig. 7). The amplitude of the effect of lovastatin increased with the time of incubation and was maximum after 72 hr of incubation (Fig. 7). Mevalonate action was maximum after 24 hr but decreased after 72 hr. At this time, the combination of lovastatin plus mevalonate induced a greater response to SNP than mevalonate alone (Fig. 7*C*). Similar results were obtained with vessels isolated from WKY rats. Mevalonate significantly decreased the maximum response (N/m^2) to NE (3.69 ± 0.20 vs. 4.35 ± 0.25 for controls, $P < 0.05$). It also significantly increased the ED_{50} for the hormone: 28.6 ± 4.8 nM (M) vs. 1.8 ± 0.8 nM (C, $P < 0.001$). Lovastatin increased the maximum response to NE (5.97 ± 0.14, $P < 0.001$) but did not change the sensitivity (ED_{50} = 0.5 ± 0.2 nM, NS). The maximum relaxation to acetylcholine was significantly improved in M vessels (2.4% ± 2.4% of precontraction with NE) by comparison with controls (22.8% ± 5.0%, $P < 0.02$); it was significantly impaired in L vessels (58.7% ± 2.3%, $P < 0.007$). The maximum response to SNP was also impaired by pretreatment with lovastatin ($P < 0.001$, not shown) but not by mevalonate. However, the sensitivity to SNP was significantly ($P < 0.001$) increased in M vessels (not shown). The vessels incubated with a mixture of lovastatin and mevalonate had intermediate response.

Neither lovastatin, mevalonate, nor the combination of the two drugs significantly altered aortic FC, PL, or the molar ratio FC/PL by comparison with controls. Aortic FC levels (μg/100 mg of wet weight) were 149 ± 24 (L), 174 ± 19 (M), 170 ± 25 (L plus M), and 142 ± 10 (C). Aortic PL levels (μg/100 mg of wet weight) were 23.3 ± 1.5 (L), 21.2 ± 0.9 (M), 19.2 ± 0.6 (L plus M), and 22.5 ± 1.1 (C). Finally, the molar FC/PL ratios were 0.54 ± 0.12 (L), 0.68 ± 0.08 (M), 0.72 ± 0.13 (L plus M), and 0.48 ± 0.03 (C).

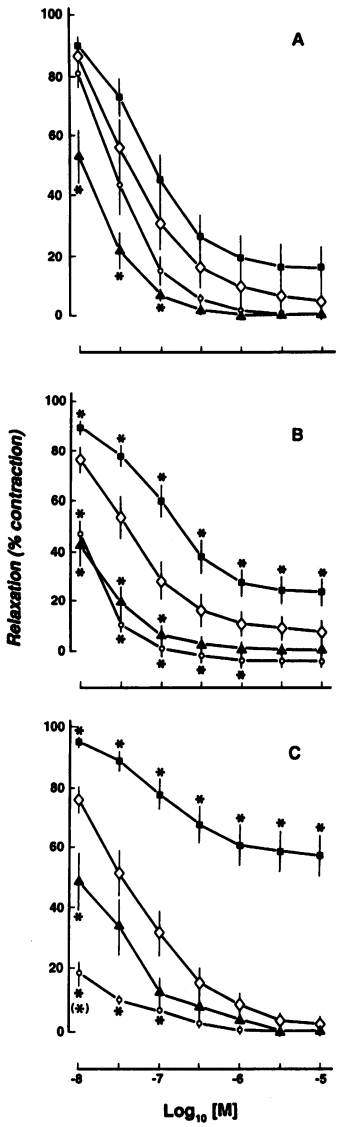

FIG. 7. Reactivity to SNP of aorta rings (Wistar rats, n = 8) incubated for 24 (*A*), 48 (*B*), and 72 (*C*) hr in DMEM containing mevalonate (10 mM, ▲), lovastatin (5 μg/ml, ■), mevalonate plus lovastatin (○), or vehicle (◇). * and (*) denote statistical difference with vehicle- and lovastatin-treated preparations, respectively.

DISCUSSION

To our knowledge, neither the hypotensive effect of mevalonate nor the hypertensive effect of lovastatin has been reported previously. Used extensively for the treatment of human hypercholesterolemia, lovastatin is perceived to have few side effects and no significant action on systemic BP, even when administered at the dose used in our experiments.

Our *in vivo* data indicate that pharmacological stimulation and inhibition of the mevalonate pathway are associated with reciprocal changes in arterial pressure. We believe these findings reflect the impact of mevalonate availability, not plasma cholesterol, on BP regulation since the modifications of BP occurred without significant change in plasma cholesterol levels. The absence of cholesterol-lowering effect of lovastatin observed in our study is in agreement with previous reports in rodents (19, 20) and contrasts with the effect seen in human subjects. Greater induction of hepatic HMG-

CoA reductase after lovastatin treatment and lower plasma levels of low density lipoproteins in rats than in humans are thought to be responsible of the species difference. We think that the choice of the rat model allowed us to unmask the regulatory role of the endogenous cholesterol pathway with lovastatin, because dissociation between exogenous and endogenous cholesterol pathway was achieved. The relevance of our findings with lovastatin to the human situation needs to be addressed in appropriate drug-intervention studies using subjects with normal or moderately elevated cholesterol levels.

Results from the *ex vivo* experiments support the hypothesis that lovastatin-induced increase in systemic BP can be attributed, in part, to drug-induced alterations of vascular reactivity, including increased response to vasoconstrictors and impairment of vasorelaxation. The mevalonate effect on BP was not associated with any significant changes in vascular contractility or relaxation. Either mevalonate influenced other pressure-regulatory system(s) or the vascular changes were too small to be detected in single isolated resistance vessels but were large enough to induce a decrease in systemic BP when summed across the resistance vascular bed. A high metabolic turnover and rapid clearance of mevalonate by the vessels could also account for the absence of measurable impact on vascular reactivity 24 hr after the last gavage. Each of these hypotheses merits experimental demonstration.

The *in vitro* data indicate that stimulation and inhibition of the mevalonate pathway are associated with reciprocal changes in the vascular reactivity to vasoconstrictors and vasodilators, thus confirming *in vivo* or *ex vivo* results. Similar results were found in WKY and Wistar rats, suggesting that the regulatory role of mevalonate is common to different strains of rats. The concentrations of lovastatin (5 μg/ml) and mevalonate (10 mM) used *in vitro* are not physiological; however, they have been commonly used for the pharmacological exploration of the cholesterol pathway in cultured cells (12, 21). Any given action of lovastatin on the vessels was counteracted by the addition of mevalonate, pointing to mevalonate availability as a regulator of vascular reactivity. There was no evidence of a significant impact of either drug on aortic FC, PL, or FC/PL molar ratio, suggesting that the lipid composition of the plasma membrane of the smooth muscle was not significantly altered. This finding is not surprising in the light of previous reports showing that aortic cholesterol synthesis is very low compared to hepatic synthesis (19, 22) and further suggests that, in the arterial tissue, lovastatin and mevalonate regulate the metabolic availability of nonsterol product(s) of the cholesterol pathway rather than cholesterol itself.

Interestingly, hypercholesterolemia and atherosclerosis are associated with vascular dysfunctions similar to the ones induced by lovastatin: increased sensitivity to adrenergic stimulation, decreased vascular response to acetylcholine, and impairment of the endothelium-independent relaxation (23–27). Thus, the primary inhibition of endogenous production of mevalonate by lovastatin reproduces many of the functional effects of an excess of exogenous cholesterol on the vasculature. We therefore propose that the systemic and vascular dysfunctions associated with atherosclerosis may be a consequence of decreased activity of the mevalonate pathway rather than simply an accumulation of cellular cholesterol as others have speculated.

The time course of the mevalonate action on acetylcholine-induced relaxation did not parallel that of SNP response (Figs. 6 and 7), suggesting that mevalonate may affect separately the release or the generation of endothelium-derived relaxing factor by endothelial cells and the endothelium-derived relaxing factor effector system of the vascular myocytes. In contrast, the time courses of the lovastatin action on endothelium-dependent and independent relaxation were parallel. Specific experiments need to be performed to evaluate separately the direct impact of both compounds on vascular myocytes and endothelial cells.

In conclusion, our study offers provocative evidence that mevalonate availability contributes to optimal regulation of cardiovascular functions. The existence of a mevalonate "box" controlling vascular tone raises the possibility of pathological dysregulation of mevalonate production contributing to essential hypertension and calls for further work to identify the specific metabolic steps involved in this cardioregulatory process. Finally, our experiments suggest that the current widespread use of HMG-CoA reductase inhibition for the control of plasma cholesterol concentrations in humans with compounds such as lovastatin may have unrecognized and deleterious effects on the regulation of arterial pressure, a possibility that must be addressed in the future by appropriate clinical studies.

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