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Effect of simvastatin on vascular smooth muscle responsiveness: involvement of Ca^{2+} homeostasis

María Álvarez de Sotomayor *, Concepción Pérez-Guerrero, María Dolores Herrera, Elisa Marhuenda

Department of Pharmacology, Faculty of Pharmacy, University of Seville, C / Profesor Garcia-Gonzalez s / n, 41012 Seville, Spain

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Abstract

This report is focused on the study of simvastatin-induced relaxation of rat aorta through its effects on vascular smooth muscle and Ca^{2+} signalling. The presence of endothelium affected only the simvastatin-induced relaxation of aortic rings precontracted with noradrenaline, but not by depolarization with KCl 80 mM. Blockade of Ca^{2+} entry through voltage-operated Ca^{2+} channels (VOCCs) by diltiazem abolished the endothelium-dependent and direct relaxation, whereas Ca^{2+}-ATPase inhibition by cyclopiazonic acid (3×10^{-5} M) only affected the endothelium-dependent relaxation. In KCl-depolarised arteries concentration–response curves for $CaCl_2$ were shifted to the right in the presence of simvastatin (3×10^{-6} and 3×10^{-5} M) or diltiazem (10^{-6} and 10^{-7} M). The transient contraction caused by noradrenaline in Ca^{2+}-free medium, which is mainly due to intracellular Ca^{2+} release, was inhibited by simvastatin (3×10^{-5} M) or cyclopiazonic acid (3×10^{-5} M) and the contraction induced by $CaCl_2$ (2×10^{-3} M) added after noradrenaline was inhibited by diltiazem and simvastatin. All the reported effects of simvastatin were inhibited by the product of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, mevalonate (10^{-3} M). These findings demonstrate that the vascular effects of simvastatin may involve both Ca^{2+} release from intracellular stores, which could promote activation of endothelial factors, and blockade of extracellular Ca^{2+} entry, which promote relaxations independent of the presence of endothelium. This action on Ca^{2+} could be related to the inhibition of isoprenoid synthesis, which subsequently affects the function of G-proteins involved in communication among intracellular Ca^{2+} pools and capacitative Ca^{2+} entry.

Keywords: HMG-CoA reductase; Simvastatin; Ca^{2+}; Ca^{2+}-ATPase; Diltiazem, Cyclopiazonic acid

1. Introduction

Simvastatin is a drug widely used in the treatment of hypercholesterolemia. This drug is an inhibitor of the rate-determining enzyme in the biosynthesis of cholesterol, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, and has proved useful in the reduction of plasma low-density lipoprotein (LDL) levels (Mauro and MacDonald, 1991). Clinical trials have demonstrated that inhibitors of HMG-CoA reductase reduce cardiovascular-related morbidity and mortality in patients with and without coronary artery disease (Scandinavian Simvastatin Survival Study Group, 1994), and that endothelium-mediated responses are improved in arteries from patients treated with HMG-CoA reductase inhibitors (Treasure et al., 1995). This improvement persists with continued administration of simvastatin despite the absence of a further reduction in serum cholesterol levels (O'Driscoll et al., 1997).

HMG-CoA reductase is also involved in the biosynthetic pathway of isoprenoids from mevalonate (Goldstein and Brown, 1990). Isoprenoids have been shown to play a role in the mechanisms leading to vascular smooth muscle cell proliferation and migration. Thus, the antiatherosclerotic effect of simvastatin might in part result from its inhibitory effect on the synthesis of isoprenoids, independently of its hypocholesterolemic properties (Soma et al., 1995; Raiteri et al., 1997). These actions of HMG-CoA reductase inhibitors on the proliferation and migration of vascular smooth muscle cells are related to Ca^{2+} signalling, which is affected by isoprenoids (Ng et al., 1994; Escobales et al., 1996). Clinical benefits of simvastatin therapy have been reported as being best explained by their direct effects on each component of the triad and on

* Corresponding author. Tel.: +34-5-4556720; fax: +34-5-4233765.
E-mail address: aldesoto@fafar.us.es (M. Álvarez de Sotomayor).

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atherosclerotic and thrombotic mechanisms within arteries, as well as through the more conventionally accepted way of decreasing plasma LDL concentrations (Vaughan et al., 1996).

When vascular and endothelial dysfunctions other than atherosclerosis are considered, chronic treatment with simvastatin is able to restore the endothelial response to acetylcholine and to normalise responses to the Na^+–K^+ ATPase inhibitor, ouabain (Alvarez de Sotomayor et al., 1999). This study was carried out with spontaneously hypertensive rats, which are resistant to the development of hypercholesterolemia (Lindberg et al., 1995). This animal model also has some differences in Ca^{2+}-signalling (Wang et al., 1995; Rahmani et al., 1999) that could contribute to explain the differences observed between hypertensive and normotensive rats after chronic treatment with simvastatin.

Not only chronic treatment, but also acute exposure to simvastatin is able to induce partly endothelium-dependent relaxation in rat aorta and small mesenteric arteries (Alvarez de Sotomayor et al., 2000) and lovastatin, another HMG-CoA reductase inhibitor, decreases blood pressure and has vasodilatatory effects in rat isolated thoracic aorta (Bravo et al., 1998).

The aim of the present study is to clarify the mechanism of the vascular effect of simvastatin with special attention being paid to the involvement of Ca^{2+} signalling in simvastatin-induced relaxation in rat aorta.

2. Material and methods

2.1. Animals

Male Wistar rats (12–14 weeks old), weighting 250–300 g, and fed on standard rat chow with free access to drinking water, were used for this study. The animals were killed by cervical dislocation and the aortae were rapidly dissected.

2.2. Aortic ring preparation

The descending thoracic aorta was placed in a modified Krebs–Henseleit solution (PSS) containing (mM): NaCl 118, KCl 4.75, $NaHCO_3$ 25, $MgSO_4$ 1.2, $CaCl_2$ 1.8, KH_2PO_4 1.2 and glucose 11. After excess fat and connective tissue were removed, the aortae were cut into 2- to 3-mm rings. Aortic rings were mounted under a basal tension of 2 g in 20-ml organ baths containing PSS and attached to a isometric transducer (Harvard UF-1), the signal was recorded by a Powerlab® data acquisition system (AD-Instruments). The tissue bath was maintained at 37 °C and bubbled with a 95% O_2–5% CO_2 gas mixture. In some experiments, the endothelium was mechanically removed by gently rubbing the intimal surface. The absence of endothelium was confirmed by the absence of relaxing effects of acetylcholine (10^{-6} M) in aortic rings previously contracted by noradrenaline (10^{-5} M). Each preparation was allowed to equilibrate for at least 90 min prior to initiation of experimental procedures, and during this period the incubation medium was changed every 20 min. For Ca^{2+}-free PSS, $CaCl_2$ was omitted and EGTA 0.5 mM was added 10 min before the start of the experiments.

2.3. Relaxant effect of simvastatin

The relaxant effect of simvastatin was assessed by adding cumulative concentrations of this drug (10^{-6} to 3×10^{-4} M) to aortic rings precontracted by noradrenaline 10^{-6} M or KCl 80 mM. This experiment was carried out in both intact and endothelium-denuded arteries, and in the presence and absence of mevalonate (10^{-3} M).

The influence of voltage-operated Ca^{2+} channels (VOCCs) in the relaxant effect of simvastatin was investigated by incubating the arteries with diltiazem (10^{-6} M) 20 min before they were contracted with noradrenaline.

Sarcoplasmic reticulum Ca^{2+}-ATPase involvement was investigated by using cyclopiazonic acid (3×10^{-5} M), added to bath at the same time as noradrenaline.

In another set of experiments, either simvastatin (3×10^{-5} M) or diltiazem (10^{-6} M) was added to the organ chamber once a stable contraction in response to KCl 80 mM was obtained. In order to test if the relaxant effect of simvastatin was counteracted by increasing Ca^{2+} concentration, extra $CaCl_2$ (2 mM) on top of the 1.8 mM that was already present in the high K^+ solution was added once the relaxation had stabilised.

2.4. Effect of simvastatin on contraction of aortic rings

In order to study the effect of simvastatin on Ca^{2+} release and on the Ca^{2+} entry components of contraction, aortic rings were incubated in Ca^{2+}-free medium and challenged with noradrenaline at 10^{-6} M, a concentration that induced a transient contraction. After relaxation of this transient contraction, $CaCl_2$ (2×10^{-3} M) was added in the continuous presence of the agonist. This experiment was also carried out following prior incubation with simvastatin (3×10^{-5} M), simvastatin (3×10^{-5} M) plus mevalonate (10^{-3} M), cyclopiazonic acid (3×10^{-5} M) and diltiazem (10^{-6} M), added to the bath 15 min before noradrenaline.

To assess the effect of simvastatin on the influx of Ca^{2+} through VOCCs, concentration–response curves for $CaCl_2$ (10^{-4} to 3×10^{-2} M) were constructed. Arteries were incubated in Ca^{2+}-free medium (without EGTA) and then exposed to a single dose of KCl (80 mM). $CaCl_2$ was then added to this solution in progressively increasing concentrations. The tissue was then washed for 1 h with Ca^{2+}-free PSS. Simvastatin (3×10^{-6} and 3×10^{-5} M) or diltiazem (10^{-6} and 10^{-7} M) was added 15 min before

the protocol was repeated. It was also repeated in the absence of any drug to obtain a control concentration–response curve.

Cyclopiazonic acid (3×10^{-5} M) added to arteries incubated in PSS containing Ca^{2+} produced a transient contraction. In another aortic ring obtained from the same animal, the cyclopiazonic acid-induced contraction was recorded in the presence of simvastatin (3×10^{-5} M) after a 15-min preincubation.

2.5. Chemical reagents and drugs

The following drugs were used: acetylcholine chloride, cyclopiazonic acid, diltiazem, EGTA, mevalonic acid lactone (mevalonate) and noradrenaline bitartrate, which were all purchased from Sigma (St. Louis, MO, USA). Simvastatin was generously provided by Merck laboratories (New Jersey, USA). All drugs were dissolved in distilled water except cyclopiazonic acid, mevalonate and simvastatin, which were dissolved in dimethylsulfoxide (DMSO). The final DMSO (less than 0.001%) concentration did not significantly affect the results. Ascorbic acid (10^{-4} M) was added to the noradrenaline.

2.6. Statistical analysis

Results are expressed as percentages from the initial precontraction level and as means ± S.E.M. (n represents the number of rats). Eight preparations were studied from a single aorta. Analysis of variance (ANOVA) and Tukey's Multiple Comparison test were used for statistical analysis. $P < 0.05$ values were considered to represent a significant difference. All curves were fitted by a concentration–response non-linear regression equation.

3. Results

3.1. Effects of simvastatin on contractions induced by noradrenaline and depolarization with KCl 80 mM

Simvastatin was able to relax aortic rings precontracted by depolarization independently of the presence of the endothelium. This effect was significantly greater than that observed in endothelium-denuded arteries precontracted with noradrenaline 10^{-6} M (Fig. 1A). In aortic rings precontracted by noradrenaline 10^{-6} M, the presence of endothelium significantly enhanced the relaxant response to simvastatin (Fig. 1B). Mevalonate significantly decreased the relaxation induced by simvastatin in aortic rings precontracted by both noradrenaline and KCl 80 mM. It is interesting that, in arteries with an intact endothelium, mevalonate was more effective in reducing the relaxation in noradrenaline-contracted arteries than in KCl 80 mM-contracted arteries ($P < 0.05$).

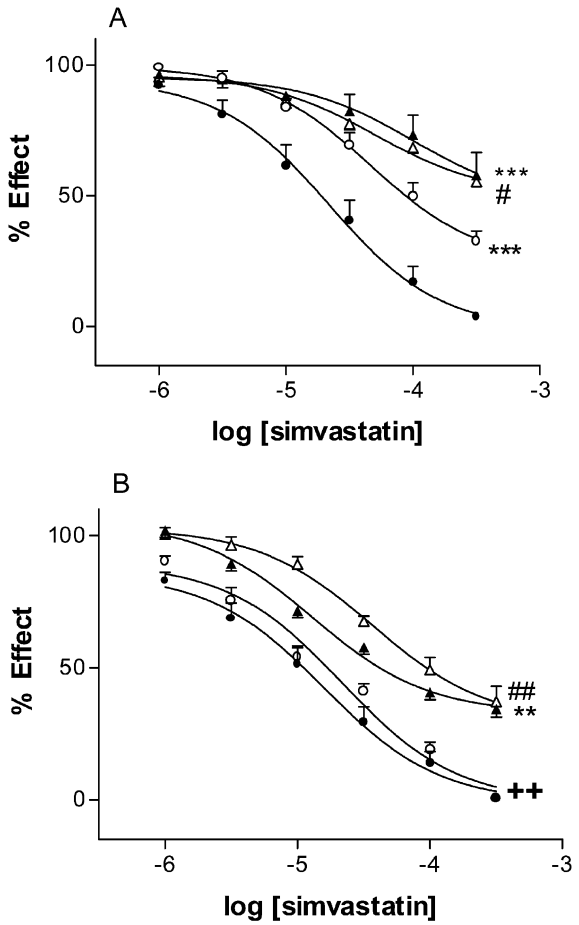

Fig. 1. Effect of simvastatin (SV, 10^{-6} M to 3×10^{-4} M) in aortic rings ($n = 6$ in each case, mean ± S.E.M.) precontracted by NA (A) or by depolarization KCl 80 mM (B). Control intact endothelium (●) or denuded (○) arteries. In the presence of mevalonate (MV 10^{-3} M) with endothelium (▲) or without endothelium (△). * * Statistically significant differences $P < 0.01$; * * * $P < 0.001$ vs. control with endothelium. #$P < 0.05$; ##$P < 0.01$ vs. control without endothelium. + + $P < 0.01$ vs. control without endothelium in aortic rings precontracted by NA.

3.2. Effect of Ca^{2+}-ATPase inhibition and Ca^{2+} antagonism on simvastatin-induced relaxations

Inhibition of sarcoplasmic reticulum Ca^{2+}-ATPase with cyclopiazonic acid (3×10^{-5} M) was able to significantly decrease the relaxant effect of simvastatin in aortic rings with endothelium. The presence of cyclopiazonic acid (3×10^{-5} M) only inhibited the endothelium-independent component of the relaxation at the highest concentration of simvastatin assayed ($P < 0.05$) (Fig. 2).

Under resting tension, cyclopiazonic acid (3×10^{-5} M) caused a transient contraction (50.39 ± 3.29% of the maximal noradrenaline-induced contraction) of endothelium-denuded aortic rings, an effect which was significantly diminished ($P < 0.001$) (12.24 ± 2.50%) after preincubation with simvastatin (3×10^{-5} M) (Fig. 3).

When the VOCCs were blocked with the Ca^{2+} channel antagonist diltiazem (10^{-6} M), both endothelium-depen-

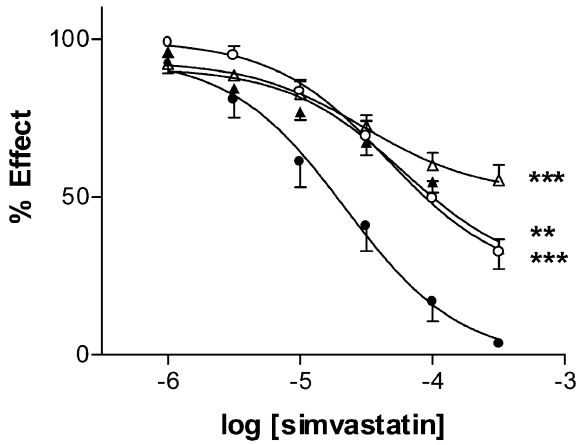

Fig. 2. Effect of simvastatin (SV, 10^{-6} to 3×10^{-4} M) in aortic rings ($n=6$ in each case, mean ± S.E.M.) precontracted by NA. Control intact endothelium (●) or denuded (○) arteries. In the presence of CPA (3×10^{-5} M) with endothelium (▲) or without endothelium (△). ** Statistically significant differences $P<0.01$; *** $P<0.001$ vs. control with endothelium.

dent and direct simvastatin-induced relaxations were significantly inhibited (Fig. 4). The presence of diltiazem at the concentration selected (10^{-6} M) did not affect significantly the precontraction elicited by noradrenaline.

3.3. Effect of simvastatin on Ca^{2+} release and Ca^{2+} entry

In Ca^{2+}-free medium, noradrenaline (10^{-6} M) elicited a transient contraction which was significantly inhibited in the presence of cyclopiazonic acid (3×10^{-5} M) ($P<0.001$) and simvastatin (3×10^{-5} M) ($P<0.05$). Diltiazem (10^{-6} M) did not affect the transient response to noradrenaline.

$CaCl_2$ (2×10^{-3} M) added after relaxation of the transient response to noradrenaline (10^{-6} M) evoked a stable contractile response, which was significantly reduced when diltiazem (10^{-6} M) ($P<0.001$) or simvastatin (3×10^{-5} M) ($P<0.01$) was present in the medium (Fig. 5A and B).

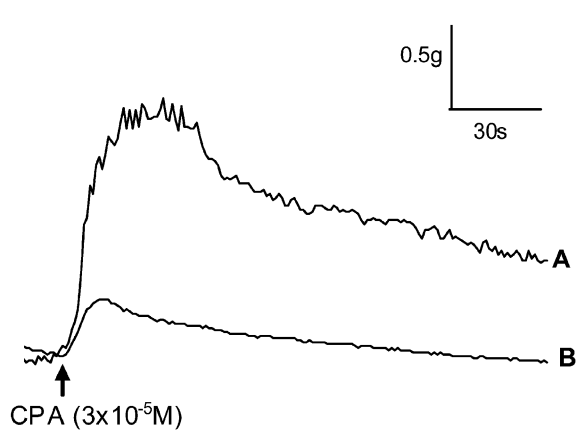

Fig. 3. Original trace of contraction induced by cyclopiazonic acid (3×10^{-5} M) under resting tension (A) and after a 15-min preincubation with simvastatin (3×10^{-5} M) (B). $n=6$ obtained from different animals.

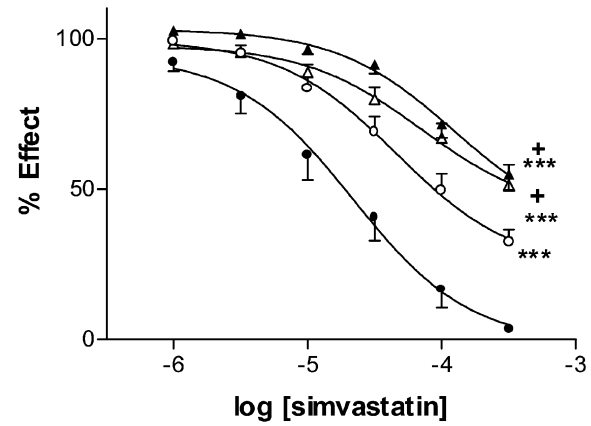

Fig. 4. Effect of simvastatin (SV, 10^{-6} to 3×10^{-4} M) in aortic rings ($n=6$ in each case, mean ± S.E.M.) precontracted by NA. Control intact endothelium (●) or denuded (○) arteries. In the presence of diltiazem (10^{-6} M) with endothelium (▲) or without endothelium (△). *** Statistically significant differences $P<0.001$ vs. control with endothelium. + $P<0.05$ vs. control without endothelium.

The inhibitory effect of simvastatin on both components of contraction was reversed in the presence of mevalonate (10^{-3} M).

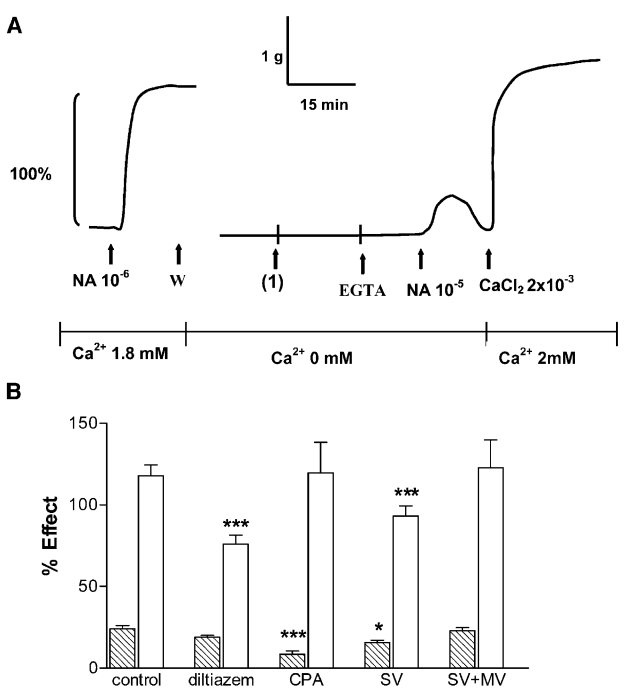

Fig. 5. Example trace of experimental procedure used to study the effect of SV, diltiazem and CPA (added at point represented by (1)) in Ca^{2+}-free-medium on noradrenaline (NA)-induced contraction and $CaCl_2$-induced contraction (A). Histograms representing contraction induced by NA (10^{-6} M) (cross-hatched bar) and $CaCl_2$ (2×10^{-3} M) (open bar) (B). The experiments represented are control and after addition of simvastatin (SV, 3×10^{-5} M), diltiazem (10^{-6} M), CPA (3×10^{-5} M) or SV plus mevalonate (MV, 10^{-3} M). Ordinate scale: contractile responses expressed as percentages of the initial contraction induced by NA (10^{-6} M) in normal Krebs. Each bar represents the mean ± S.E.M. for 6–8 experiments. * Statistically significant differences $P<0.01$; *** $P<0.001$ vs. control without endothelium.

When aortic rings were incubated in Ca^{2+}-free 80 mM KCl medium, addition of cumulative concentrations of $CaCl_2$ (10^{-4} to 3×10^{-2} M) elicited a contractile response. The concentration–response curve for $CaCl_2$ was shifted to the right by diltiazem (10^{-6} and 10^{-7} M) and simvastatin (3×10^{-5} and 3×10^{-6} M) without there being significant differences between them (Fig. 6A and B).

For further comparison of the effect of simvastatin and diltiazem, concentrations of these drugs that elicited similar shifts to the right of the $CaCl_2$ concentration–response curves were selected: 3×10^{-5} and 10^{-6} M, respectively. These concentrations of simvastatin and diltiazem were tested in denuded aortic rings precontracted by depolarization with KCl 80 mM in a medium containing 1.8 mM Ca^{2+}. Both simvastatin (3×10^{-5} M) and diltiazem (10^{-6} M) induced a similar relaxation, $71.39\pm4.48\%$, $n=6$ and $71.91\pm3.86\%$, $n=6$, respectively. The relaxant effect of diltiazem was counteracted by addition of extra $CaCl_2$ (2×10^{-3} M), reaching $72.54\pm2.12\%$. However, when relaxation was induced by simvastatin (3×10^{-5} M), the addition of extra $CaCl_2$ (2×10^{-3} M) could not restore the contraction (Fig. 7).

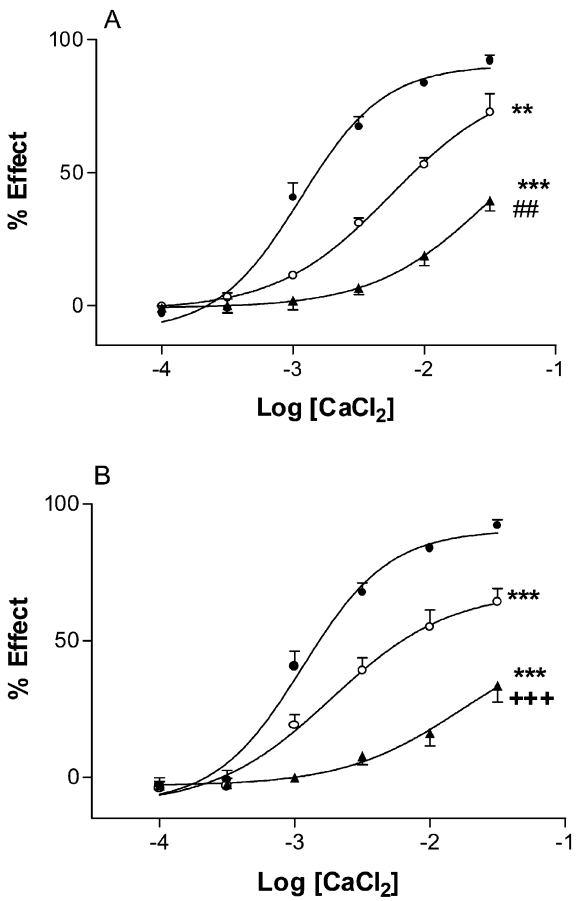

Fig. 6. Dose–response curves for $CaCl_2$ (10^{-4} to 3×10^{-2} M) in depolarized aortic rings ($n=6$ in each case, mean ± S.E.M.) incubated in Ca^{2+}-free medium. In the absence, control (●) or in the presence of diltiazem (A) 10^{-6} M (▲) or 10^{-7} M (○). In the presence of simvastatin (B) 3×10^{-6} M (○) or 3×10^{-5} M (▲). ** Statistically significant differences $P<0.01$; $^{***}P<0.001$ vs. control dose–response curve. $\#\#P<0.01$ vs. concentration–response curve in the presence of diltiazem 10^{-7} M. $+++P<0.001$ vs. concentration–response curve in the presence of SV 3×10^{-6} M.

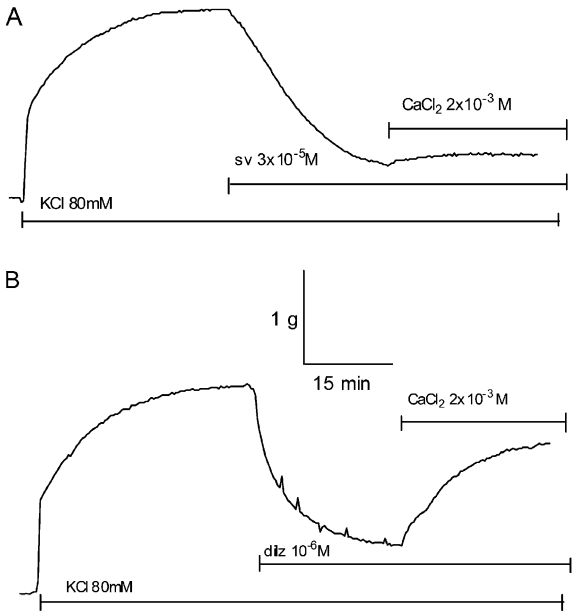

Fig. 7. Effect of simvastatin 3×10^{-5} M (A) or diltiazem 10^{-6} M (B) on denuded aortic rings ($n=6$) precontracted by depolarization with KCl 80 mM in a medium containing calcium and subsequent addition of extra $CaCl_2$ (2×10^{-3} M).

4. Discussion

Simvastatin, an inhibitor of HMG-CoA reductase, is able to relax precontracted aortic rings, this effect being partially dependent of the presence of the endothelium in arteries precontracted by noradrenaline. It has been previously demonstrated in endothelium-intact aortic rings that the simvastatin-induced relaxation is partially mediated by the release of endothelial factors (Alvarez de Sotomayor et al., 2000). However, there was a direct effect of simvastatin on vascular smooth muscle that remained to be analysed. The aim of the present study was to clarify the mechanism involved in the direct vascular smooth muscle effect of simvastatin with respect to Ca^{2+} signalling, as well as to consider the involvement of Ca^{2+} signalling in the endothelial response.

Simvastatin was able to relax aortic rings contracted by depolarization with KCl 80 mM and subsequent opening of VOCCs (Marriot, 1988), suggesting that voltage-operated Ca^{2+} entry may be altered in response to simvastatin. To confirm this hypothesis, we verified that diltiazem, an antagonist of VOCCs, reduced the relaxant effect of simvastatin on noradrenaline-precontracted aortic rings. The inhibition of the simvastatin-induced relaxation in endothe-

lium-denuded arteries suggested that blockade of Ca^{2+} entry is involved in the effect of simvastatin in vascular smooth muscle. In the same way, simvastatin and diltiazem were also able to shift to the right the $CaCl_2$ concentration–respose curves in KCl-depolarised arteries. These results agree with those of previous studies that reported a blockade of L-type Ca^{2+} channels by simvastatin in rat islet β-cells. (Yada et al., 1999). However, while the relaxant effect induced by diltiazem was counteracted by addition of $CaCl_2$, the relaxation due to simvastatin was not reversible, suggesting that simvastatin might indirectly inhibit Ca^{2+} influx or have some other effect on Ca^{2+} homeostasis. The endothelium-dependent relaxation in response to simvastatin was also inhibited when VOCCs were blocked with diltiazem. This endothelium-dependent relaxant effect is known to be mediated by the release of endothelial factors such as nitric oxide and cyclo-oxygenase products (Alvarez de Sotomayor et al., 2000). Blockade of Ca^{2+} entry might affect to the release and/or effect of endothelial factors (Griffith et al., 1986).

In order to assess the involvement of depletion of intracellular Ca^{2+} stores and subsequent Ca^{2+} entry across the plasma membrane in the vascular effect of simvastatin, the noradrenaline-induced contraction in Ca^{2+}-free medium and the subsequent contractile response to $CaCl_2$ were studied following pre-incubation with simvastatin. The results showed that simvastatin significantly inhibited both components of contraction. In contrast, diltiazem only affected Ca^{2+} entry and cyclopiazonic acid, the Ca^{2+}-ATPase inhibitor, only decreased the noradrenaline-induced contraction. These results suggest that simvastatin could affect Ca^{2+} release from intracellular pools, as has been previously described in vascular smooth muscle cells (Ng et al., 1994; Escobales et al., 1996), and capacitative Ca^{2+} entry, as confirmed by the inhibition of contraction due to $CaCl_2$ after noradrenaline stimulation. Although decreased responses to phenylephrine in Ca^{2+}-free medium and inhibition of intracellular Ca^{2+} release in the presence of simvastatin have been previously described (Eatman et al., 1998; Tesfamarian et al., 1999), our results also showed, for the first time, a decrease in contraction in response to $CaCl_2$ in depolarised arteries, which is mainly due to Ca^{2+} entry through VOCCs.

Simvastatin is an inhibitor of HMG-CoA reductase that not only decreases the endogenous synthesis of cholesterol, but also reduces the intracellular concentration of isoprenoids. Some of the properties of simvastatin, including its antiproliferative, anti-oxidant and Ca^{2+} mobilisation effects have been reported to be the consequence of its inhibitory effect on the synthesis of isoprenoid intermediates, such as dolichol, ubiquinone and farnesyl pyrophosphate (Goldstein and Brown, 1990; Doyle et al., 1993; Raiteri et al., 1997). Indeed, isoprenylation (with farnesyl or geranyl–geranyl chains) is one post-translational modification of proteins such as the gamma subunit of heterotrimeric G (Fukada et al., 1990), Rho (Casey et al., 1994) and p21 Rac (Lin et al., 1996), proteins that may be involved in the effects of simvastatin. Small G proteins, which are sensitive to prenylation, have been shown to be involved in capacitative Ca^{2+} entry. Moreover, one of the steps linking the depletion of intracellular Ca^{2+} pools to Ca^{2+} entry across the plasma membrane requires the hydrolysis of GTP and may involve a small G protein (Bird and Putney, 1993), and there is also evidence that G proteins are involved in communication among intracellular Ca^{2+} pools (Gill et al., 1986; Ghosh et al., 1989).

The effects on Ca^{2+} signalling elicited by simvastatin could affect vascular responsiveness and help explain some of the beneficial cardiovascular effects of HMG-CoA reductase inhibitors that are not related to their lipid-lowering properties. In patients treated with a therapeutic dose of HMG-CoA reductase inhibitors (from 20 to 60 mg daily), the plasma concentration of simvastatin ranges between 0.01 and 1 μM (Laufs et al., 1998; Lilja et al., 1998; Kantola et al., 1998). Thus, a concentration that could improve vasomotion by both promoting endothelium-dependent and vascular smooth muscle relaxation might be achieved in the plasma of patients receiving high therapeutic dose of simvastatin.

Another important result was that when aortic rings were precontracted by noradrenaline, the relaxant effect of simvastatin was significantly amplified by the presence of endothelium. The endothelium-dependent relaxation was reduced by incubating the aortic rings with cyclopiazonic acid, which suggests that the effects of simvastatin on the Ca^{2+} homeostasis of endothelial cells might contribute to vasodilation. In blood vessels, Ca^{2+} release and the subsequent increase in cytosolic Ca^{2+} concentration in endothelial cells could activate nitric oxide synthase and induce nitric oxide-mediated relaxation of aortic rings (Lückoff et al., 1988; Xiu-Fong et al., 1994). Nitric oxide and other vasoactive agents from endothelial cells could mask any direct effects of the Ca^{2+}-ATPase inhibitors on smooth muscle (Gibson et al., 1998). This fact could also help explain the different relaxant effect elicited by simvastatin in noradrenaline and KCl 80 mM-precontracted aortic rings, since the contribution of Ca^{2+} release from intracellular Ca^{2+} stores is more important in the contraction induced by noradrenaline than in that induced by depolarization (Minneman, 1988).

Incubation with mevalonate, the product of HMG-CoA reductase, reduced all the effects of simvastatin described in this paper. This result indicates that both the relaxation and the inhibition of the contraction obtained by using simvastatin are mediated by its inhibition of HMG-CoA reductase. Interestingly, mevalonate was less effective in inhibiting the relaxant effect of simvastatin on arteries contracted by depolarization and subsequent Ca^{2+} entry through VOCCs than it was on aortic rings precontracted by noradrenaline. Preincubation with mevalonate also restored the $CaCl_2$-induced contraction after stimulation with noradrenaline, which is mainly the consequence of the

activation of G protein-coupled adrenergic receptors and the release of Ca^{2+} from intracellular stores, an effect which is sustained by the secondary activation of VOCCs (Nelson et al., 1988; Missiaen et al., 1992). These results suggest that simvastatin might act at the level of G-proteins by reducing the isoprenoid concentration and thus interfering with their post-translational modification.

In summary, simvastatin is able to produce vascular relaxation independent of its lipid lowering properties by acting on smooth muscle and endothelium of rat isolated aorta. The effect of simvastatin on vascular smooth muscle may involve both Ca^{2+} release from intracellular stores and blockade of extracellular Ca^{2+} entry. This action on Ca^{2+} signalling could be related to the inhibition of isoprenoid synthesis, which affects the function of G-proteins involved in communication among intracellular Ca^{2+} pools and capacitative Ca^{2+} entry. Because of these actions, simvastatin could have a potential beneficial effect on some cardiovascular pathologies associated with vasoconstriction.

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