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Simvastatin evokes an unpredicted inhibition of β-adrenoceptor-mediated vasodilatation in porcine coronary artery

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ABSTRACT

HMG-CoA reductase inhibitors, or statins, are widely used as cholesterol-lowering agents in the treatment of dyslipidemias. Statins have also been reported to have pleiotropic effects, independent of their effects on cholesterol synthesis, possibly through inhibition of the monomeric G proteins Ras and Rho, which are able to signal through ERK and Rho kinase activities, respectively. We have previously demonstrated that inhibition of ERK activation enhances β-adrenoceptor-mediated vasodilatation in the porcine isolated coronary artery. As statins can also inhibit ERK activation, the initial aim of this study was to determine whether statins have a similar influence on β-adrenoceptor-evoked vasodilatation. Segments of porcine distal coronary artery were mounted in a Mulvany wire myograph and bathed in Krebs–Henseleit buffer gassed with 95% O_2/5% CO_2 and maintained at 37 °C. Tissues were pre-contracted with the thromboxane mimetic U46619 prior to cumulative concentration–response curves to the β-adrenoceptor agonist salbutamol in the absence or presence of simvastatin (1, 5 or 10 μM), pravastatin (10 μM), or lovastatin (10 μM). Simvastatin inhibited the salbutamol-induced relaxation of the coronary artery. Similar effects were seen with lovastatin, but not pravastatin or the sodium salt of simvastatin. Simvastatin, but not pravastatin also inhibited the relaxations to the Ca^{2+}-activated K^+ channel opener NS1619 and the K_{ATP} channel opener pinacidil. Unexpectedly, these data indicate that, rather than enhancing β-adrenoceptor-mediated vasodilatation, lipophilic statins impair these responses. This is likely to be due to effects on K^+ channels.

1. Introduction

Statins are widely-used drugs for the treatment of hypercholesterolaemia. By inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, statins prevent the conversion of HMG-CoA to mevalonic acid, an early precursor of cholesterol, thereby inhibiting the biosynthesis of cholesterol. Statins have also been reported to have pleiotropic effects, independent of a reduction in cholesterol levels and these effects may underlie the ability of statins to improve cardiovascular health in patients without raised plasma cholesterol (Bonetti et al., 2003).

The pleiotropic effects of statins could be due to a reduction in the activity of other intracellular signalling pathways. In addition to lowering cholesterol, statins also decrease levels of intermediates of the mevalonate pathway, including the isoprenoids farnesyl pyrophosphate and geranylgeranyl pyrophosphate. Both of these isoprenoids are involved in post-translational modification of multiple proteins, including the monomeric G proteins Ras and Rho. This post-translational modification enables translocation of the monomeric G proteins to the cell membrane, where they are activated (Lane and Beese, 2006). For example, simvastatin inhibits proliferation of human smooth muscle cells by preventing the farnesylation of Ras (Negre-Aminou et al., 1997).

The regulation of vascular smooth muscle tone is a complex process based on the balance between vasoconstricting and vasodilating substances. Activation of Rho kinase and extracellular signal-regulated kinase (ERK), for example, is associated with vasoconstriction (Roberts, 2004), whilst on the other hand, increases in the levels of nitric oxide result in vasodilatation. Since Rho and Ras are upstream of Rho kinase and ERK activities, respectively, statins would be expected to inhibit vasoconstriction. Previous studies have demonstrated that lipophillic statins, including simvastatin and lovastatin induce an acute relaxation of isolated blood vessels directly (Bergdahl et al., 2003; Mraiche et al., 2005; Nagaoka et al., 2007). On the other hand, hydrophilic statins such as pravastatin do not induce relaxation (Bergdahl et al., 2003; Mraiche et al., 2005).

We have previously shown that inhibition of ERK activation results in the enhancement of β-adrenoceptor-mediated

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vasodilatation (Uhiara et al., 2009), suggesting that ERK activity negatively regulates β-adrenoceptor function. As statins have been shown to inhibit ERK activation in vascular smooth muscle (Tristano et al., 2007), the initial aim of this investigation was to determine whether simvastatin also enhance β-adrenoceptor-mediated vasodilatation.

2. Methods

2.1. Tissue preparation

Hearts from pigs of both sexes were obtained from a local abattoir and transported back to the laboratory in ice-cold Krebs–Henseleit buffer, previously gassed with 95% O_2, 5% CO_2. The composition of Krebs–Henseleit solution, in mM, was as follows: NaCl, 128; KCl, 4.8; $MgSO_4$, 1.1; $NaHCO_3$, 25; KH_2PO_4, 1.2; D-glucose, 12; $CaCl_2$, 1.25. Anterior descending coronary arteries were then dissected out from the hearts as previously described (Uhiara et al., 2009). The vessels were stored overnight at 4 °C in Krebs–Henseleit solution containing Ficoll (2%) pre-gassed with 95% O_2, 5% CO_2. Previous experiments have demonstrated that these conditions have negligible effect on contractile and vasodilator function in isolated blood vessels (Lot and Wilson, 1994).

The following day, distal sections of coronary arteries were dissected into 2 mm ring segments and mounted on a Mulvany, four-channel wire myograph attached to a PC computer via a PowerLab data acquisition system (ADInstruments Ltd., UK). The 5 mL baths contained Krebs–Henseleit solution maintained at 37 °C and gassed with 95% O_2, 5% CO_2. A tension of 2.5 g was applied to each ring segment prior to investigation. In some experiments, the endothelium was removed by rubbing the lumen of the arteries with a fine pair of forceps prior to mounting in the myograph.

2.2. Experimental procedure

After challenging artery rings to KCl (60 mM) as previously described (Uhiara et al., 2009), the rings were incubated in Krebs–Henseleit buffer for 45 min in either the absence or presence of various statins: simvastatin (1 μM, 5 μM or 10 μM), lovastatin (10 μM), pravastatin (10 μM) and simvastatin Na^+ (5 μM). Previous studies have demonstrated that simvastatin and lovastatin have an acute effect on vascular responses in isolated blood vessels at these concentrations (Bergdahl et al., 2003; Mraiche et al., 2005; Nagaoka et al., 2007). Control tissues received vehicle only (0.1% v/v dimethyl sulphoxide (DMSO) for 10 μM lovastatin and 0.05% v/v DMSO for 5 μM simvastatin). The tissues were precontracted to approximately 65–80% of the peak KCl contractile response using the thromboxane mimetic U46619 (10–50 nM), before relaxations were induced using cumulative concentrations of the β_2-adrenoceptor agonist salbutamol (10 nM–30 μM). In a further set of experiments, forskolin (1 nM–3 μM) was applied to U46619-pre-contracted vessels to assess receptor-independent vasodilatation. In order to assess the effect of statins on K^+ channel-dependent responses, relaxations were induced using cumulative concentrations of the selective opener of large-conductance calcium-activated K^+ channels (BK_{Ca}) NS1619 (10 nM–30 μM) or the selective opener of ATP-sensitive K^+ channels (K_{ATP}) pinacidil (1 nM–30 μM). Alternatively, tissues were incubated with simvastatin (5 μM) after pre-contraction with KCl.

In all experiments there was no significant difference between the levels of pre-contraction evoked by U46619 in vehicle- and drug-exposed vessel segments.

2.3. Statistical analyses

The data were analysed using the computer program Prism (GraphPad Software, Inc., La Jolla, CA, USA). Relaxations were expressed as a percentage change from the U46619-induced contraction and were expressed as means ± S.E.M of n experiments, where n represents the number of different animals. Statistical comparisons were made using ANOVA followed by a Bonferroni post-hoc test. Unpaired Student's t-tests were used to assess differences between the levels of U46619-evoked pre-contraction. A P-value < 0.05 was considered statistically significant.

2.4. Reagents and compounds

9,11-Dideoxy-9α,11α-methanoepoxy prostaglandin $F_{2\alpha}$ (U46619), forskolin—Axxora (Bingham, Nottinghamshire, UK); salbutamol, simvastatin, lovastatin, pravastatin—Tocris Bioscience (Bristol, UK); 1,3-dihydro-1-[2-hydroxy-5-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2H-benzimidazol-2-one (NS1619), pinacidil—Sigma (Poole, Dorset, UK); simvastatin Na^+—Calbiochem (Nottingham, UK). The remaining chemicals were obtained from Sigma (Poole, Dorset, UK).

3. Results

3.1. The effect of statins on β-adrenoceptor-mediated relaxation

Cumulative addition of the β_2-adrenoceptor agonist salbutamol (10 nM–30 μM) to U46619-precontracted porcine coronary artery (PCA) segments evoked concentration-dependent relaxations with an maximum relaxation of 100 ± 3% (mean ± S.E.M.) of the U46619-induced contraction and a pEC_{50} value of 5.9 ± 0.1 (n=16). Addition of simvastatin to the tissues had no effect on basal tone. Pre-incubation with 1 μM simvastatin had no effect on the relaxations to salbutamol (Fig. 1A). On the other hand, pre-incubation with 5 μM simvastatin produced a marked inhibition of the relaxations to salbutamol (Fig. 1A). In tissues incubated with 10 μM simvastatin, there was no relaxation to salbutamol (data not shown). Similarly, lovastatin (10 μM; Fig. 1B) reduced relaxations to salbutamol, whereas neither pravastatin (10 μM; Fig. 1C) nor simvastatin Na^+ (5 μM; Fig. 1D) altered the relaxation characteristics of salbutamol. Removal of the endothelium had no significant effect on the inhibition of the salbutamol relaxations with 5 μM simvastatin (76.9 ± 4.5% relaxation to 30 μM salbutamol in control compared to 48.0 ± 5.3% relaxation in the presence of 5 μM simvastatin, n=7).

3.2. The effect of statins on forskolin-induced relaxation

β-Adrenoceptors cause relaxation of blood vessels through activation of adenylyl cyclase and an increase in cAMP. In order to determine whether simvastatin could be inhibiting the β-adrenoceptor-relaxation through inhibition of cAMP-mediated relaxations, we investigated the effect of simvastatin on the relaxation responses to the adenylyl cyclase activator forskolin. As can be seen in Fig. 2, simvastatin did not alter relaxations at any concentration of forskolin.

3.3. The effect of simvastatin on relaxations induced by K^+ channel openers

A previous study has demonstrated that simvastatin inhibits large-conductance calcium-activated K^+ channel (BK_{Ca}) activity in porcine isolated coronary artery smooth muscle cells (Seto et al., 2007). As β-adrenoceptors are able to cause relaxations