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The effects of the statins lovastatin and cerivastatin on signalling by the prostanoid IP-receptor

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1 The prostanoid-IP receptor may be unique among G protein coupled receptors in that it is isoprenylated. In this study, we investigated the effects of the statins lovastatin and cerivastatin on signalling by the mouse (m) IP and the human (h) IP receptors, over-expressed in human embryonic kidney (HEK) 293 cells and by the hIP receptor, endogenously expressed in human erythroleukaemia cells.

2 Both statins significantly reduced IP receptor-mediated cyclic AMP generation and intracellular calcium ($[Ca^{2+}]_i$) mobilization in a time and concentration dependent manner but had no effect on signalling by the non-isoprenylated β_2 adrenergic receptor or by the human prostanoid-TP receptor isoforms.

3 Cerivastatin (IC_{50}, 50–90 nM) was significantly more potent than lovastatin (IC_{50}, 0.80–4.2 μM) in inhibiting IP receptor signalling.

4 Whereas IC_{50} values indicated that the hIP receptor was significantly more sensitive than the mIP receptor to the statins, the extent of inhibition of cyclic AMP generation by the mIP receptor was significantly greater than that of the hIP receptor to either statin, even at the highest concentrations used.

5 Pretreatment with either statin significantly reduced IP receptor mediated desensitization of signalling by the h.TPα, but not by the h.TPβ, receptor isoform.

6 These data generated in whole cells point to the possibility that statin therapy may interfere with IP receptor signalling *in vivo*; such interference may be extenuated under conditions where circulating statin levels are elevated and may account, in part, for some of the pleiotropic affects of the statins not attributed solely to their lipid lowering properties.
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Abbreviations: $[Ca^{2+}]_i$, intracellular calcium; GPCR, G protein coupled receptor; HEK, human embryonic kidney; HEL, human erythroleukaemia; HMG CoA, hydroxy methyl glutaryl coenzyme A; IP receptor, prostanoid-IP receptor; PG, prostaglandin; PLC, phospholipase C; TXA_2, thromboxane A_2; TP receptor, prostanoid-TP receptor

Introduction

The prostanoid prostacyclin (prostaglandin (PG) I_2) plays a central role in the maintenance of vascular haemostasis (Narumiya *et al*., 1999). It acts as a potent inhibitor of platelet aggregation and as a vasodilator (Vane & Botting, 1995) and may confer a cytoprotective effect against tissue injury in acute myocardial ischaemia or following hypoxic exposure of vascular endothelial cells (Sakai *et al*., 1990). Prostacyclin may also be important in the regulation of renal blood flow (Negishi *et al*., 1995) and acts as a lipolytic agent in adipocytes (Chatzipanteli *et al*., 1992).

Prostacyclin signals through its specific G protein coupled receptor (GPCR), termed the prostanoid IP receptor (Coleman *et al*., 1994). The IP receptor primarily couples to activation of adenylyl cyclase (Boie *et al*., 1994), but may also couple to other effector systems including phospholipase (PL) C (Namba *et al*., 1994) and to inhibition of adenylyl cyclase (Schwaner *et al*., 1995).

Isoprenylation is a post-translational lipid modification of proteins whereby carbon (C) 15 farnesyl or C20 geranylgeranyl isoprenoids, derived from the mevalonate/cholesterol biosynthetic pathway, are attached to specific cysteine residues located in distinct carboxyl-terminal 'isoprenylation motifs' of proteins (Zhang & Casey, 1996). We recently established that the IP receptor is isoprenylated within its carboxyl terminal tail (C-tail) region (Hayes *et al*., 1999). Whereas isoprenylation is not required for ligand binding, it is absolutely required for IP receptor activation of adenylyl cyclase and for efficient coupling to PLC (Hayes *et al*., 1999).

Lipid lowering agents encompass several classes of drugs including the statins, which inhibit hydroxy methylglutaryl coenzyme A (HMG CoA) reductase, and are widely used in the treatment of hypercholesterolemia (Farnier & Davignon, 1998) and in the prevention of coronary heart disease (Thompson, 1998; Maronm *et al*., 2000; Vaughan *et al*., 2000). Reductions in low-density lipoprotein (LDL) cholesterol achieved with the statins (Hanefeld *et al*., 1999; Stein *et al*., 1999) result in significant reductions in morbidity associated with coronary artery disease (Farnier & Davignon, 1998), together with reductions in the incidence of stroke and total mortality (Sacks *et al*., 1996; MacMahon *et al*., 1998). Since mevalonate, the product of the HMG CoA reductase reaction,

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is the precursor of numerous nonsteroidal isoprenoid metabolites in addition to cholesterol, including farnesyl and geranylgeranyl pyrophos-phate, inhibition of HMG CoA reductase through statin therapy has the potential to result in pleiotropic effects not necessarily directly associated with their cholesterol lowering properties but rather may also inadvertently target isoprenylated proteins (Corsini *et al*., 1999; Guijarro *et al*., 1998). Thus, in the present study we investigated the effects of lovastatin and cerivastatin, a recently developed HMG CoA reductase inhibitor, on IP receptor function in human embryonic kidney (HEK) 293 cells stably over-expressing the mouse (m) IP and human (h) IP receptor and in the platelet-like human erythroleukaemia 92.1.7 (HEL) cells, which endogenously express the hIP receptor at high levels. Our studies confirm the functional requirement for isoprenylation by both mIP and hIP receptors and indicate that nM concentrations of cerivastatin significantly impair IP receptor signalling.

Methods

Materials

Iloprost, [^{3}H]-iloprost (15.3 Ci mmol^{-1}) and [^{3}H]-CGP-12177 (41.0 Ci mmol^{-1}) were purchased from Amersham Pharmacia Biotech. Fura2/AM was purchased from Calbiochem. Alprenolol and isoproterenol were purchased from Sigma. [^{3}H]-cyclic AMP (15–30 Ci mmol^{-1}) was purchased from American Radiochemicals Inc.

Transfections and stable cell lines

HEK.mIP and HEK.mIP^{SSLC} and HEK.β_2AR cell lines, stably over expressing the wild type mIP receptor, the isoprenylation defective mIP^{SSLC} receptor and the human β_2 adrenoceptor (β_2 AR), respectively, have been previously described (Hayes *et al*., 1999). The full-length hIP receptor cDNA was subcloned into pHM6 (Roche) to generate the plasmid pHM6:hIP. HEK.hIP cell lines stably overexpressing the wild type hIP receptor in HEK 293 cells were established, using pHM6:hIP, and characterized essentially as previously described (Hayes *et al*., 1999). HEK.TPα10 cells and HEK.TPβ3 cells over-expressing the human prostanoid thromboxane (TX) A_2 receptor (TP) α and β isoforms, respectively, have been previously described (Walsh *et al*., 1998; 2000a). Cells were transfected with pCMV:Gα_q as previously described (Kinsella *et al*., 1997).

Measurement of cytotoxicity

Cytotoxicity assays in HEL cells and HEK 293 cells preincubated for 20 h in the absence or presence of lovastatin (1 and 10 μM) or cerivastatin (50 nM and 0.5 μM) were carried out using CytoTox 96 Non-Radioactive Cytotoxicity Assay kit (Promega) essentially as described by the manufacturer.

Measurement of cyclic AMP

To investigate the effect of the statins on ligand mediated cyclic AMP generation, cells were preincubated for 16 h in the presence of lovastatin (10 μM) or cerivastatin (0.5 μM); alternatively, lovastatin (10 μM) or cerivastatin (0.5 μM) were added to cells every 24 h over a period of 96 h. For time course assays, cells were pre-incubated in the presence of lovastatin (10 μM) or cerivastatin (0.5 μM) for times ranging from 0–24 h. For concentration response studies, cells were pre-incubated with lovastatin (0–20 μM) or cerivastatin (0–5 μM) for 16 h. Thereafter, non viable cells were removed and cyclic AMP assays were performed at 37°C on viable cells as previously described (Hayes *et al*., 1999) following stimulation of cells for 10 min with the IP agonist, cicaprost (1 μM) or the β_2 AR agonists, alprenolol (10 μM) or isoprenaline (1 μM). Levels of cyclic AMP produced by ligand stimulated cells were expressed as fold stimulation over basal (fold increase $\pm$ s.e.mean, $n=4$).

Radioligand binding studies

Where specified cells were pre-incubated in the presence of 10 μM lovastatin or 0.5 μM cerivastatin for 16 h. IP receptor radioligand binding assays were carried on cell membranes prepared from viable cells in the presence of 4 nM [^{3}H]-iloprost (15.3 Ci mmol^{-1}) as previously described (Hayes *et al*., 1999). β_2 AR assays were carried out on whole cells using 25 nM [^{3}H]-CGP-12177 (41.0 Ci mmol^{-1}) at 14°C for 3 h using 100 μg protein in a final volume of 100 μl, essentially as described (Gagnon *et al*., 1998).

Measurement of intracellular Ca^{2+} mobilization

$[Ca^{2+}]_i$ measurements were made in FURA2/AM preloaded cells as previously described (Kinsella *et al*., 1997). Where specified, cells were pre-incubated for 16 h in the presence of lovastatin (10 or 1 μM) or cerivastatin (0.5 μM or 50 nM) and were stimulated with cicaprost (1 μM) or, as a control, with the TP agonist U46619 (1 μM). For concentration response studies, cells were pre-incubated with lovastatin (0–20 μM) or cerivastatin (0–5 μM) for 16 h. In experiments investigating the counter regulation of TP receptor signalling by the IP receptor, HEK.TPα10 cells and HEK.TPβ3 cells, transiently co-transfected with Gα_q, were grown for 16 h in the presence or absence of lovastatin (10 μM) or cerivastatin (0.5 μM); thereafter FURA2/AM preloaded viable cells were stimulated with the IP receptor agonist cicaprost (1 μM) followed by stimulation with the TP receptor agonist U46619 (1 μM). Data presented in the figures are representative data from at least four independent experiments and were calculated as mean changes in intracellular Ca^{2+} mobilized ($\Delta[Ca^{2+}]_i \pm$ s.e.mean, nM; $n=4$) as a function of time (seconds, s) following ligand stimulation.

Data analyses

Radioligand binding data was analysed using GraphPad Prism V2.0 programme (GraphPad Software Inc., San Diego, CA, U.S.A.). Where appropriate, inhibitory concentration (IC_{50}) values were calculated using the GraphPad Prism V2.0 programme. Briefly, sigmoidal concentration response curves were generated where X was the logarithm of statin concentration and Y was the response (either cyclic AMP fold increase or $\Delta[Ca^{2+}]_i$ mobilization, nM). IC_{50} values were determined using the equation

$$Y = \text{Bottom} + \{(\text{Top} - \text{Bottom})/1 + 10^{\log IC50-X}\}$$

where Top is the maximal response in the absence of statin, and bottom is the maximal inhibitory effect in the presence of the statins. Thereafter, data were plotted and are presented as line graphs drawn as XY scatter plots where data points were connected by smoothed lines. Statistical analyses were carried out using the unpaired Student's *t*-test using the Statworks Analysis Package. *P*-values <0.05 were considered to indicate a statistically significant difference.

Results

The effect of the statins on IP receptor-mediated cyclic AMP generation

The effects of lovastatin and cerivastatin on IP receptor signalling were initially investigated in HEL cells, which endogenously express the hIP receptor, and in HEK 293 cells stably over-expressing the mIP receptor (HEK.mIP cells) or, as a control, in HEK 293 cells over-expressing the human β_2 AR (HEK.β_2AR cells). Initial cytotoxicity studies established that $64.2 \pm 1.0\%$ ($n=8$) of HEK.mIP cells and $60.4 \pm 2.6\%$ ($n=8$) of HEL cells remained viable following 20 h exposure to either statin with no significant differences observed irrespective of the cell type or statin used. Thereafter, measurement of ligand binding and intracellular signalling was performed on viable cells only. Pre-incubation of cells with lovastatin (Table 1) or cerivastatin (0.5 μM, data not shown) for 16 h had no significant effect on [^{3}H]-iloprost binding by IP receptors expressed in HEL cells or in HEK 293 cell lines or on [^{3}H]-CGP-12177 binding by the β_2 AR expressed in HEK 293 cell lines.

In HEK.mIP cells, lovastatin (10 μM) and cerivastatin (0.5 μM) pretreatment resulted in 84.6 and 88.1% reductions in cicaprost mediated cyclic AMP generation, respectively (Figure 1). In HEL cells, lovastatin and cerivastatin pretreatment resulted in 54.1 and 56.1% reductions in cicaprost mediated cyclic AMP generation, respectively (Figure 1). It was noteworthy that the extent of inhibition of cyclic AMP generation by the mIP receptor was significantly greater than that of the hIP receptor following lovastatin ($P<0.0002$) and cerivastatin ($P<0.0001$) treatment. Neither lovastatin ($P>0.2$) nor cerivastatin ($P>0.1$, Figure 1) affected alprenolol mediated cyclic AMP generation in HEK.β_2 AR cells. Similarly, neither lovastatin (20 μM; $P>0.33$) nor cerivastatin (5 μM; $P>0.42$) affected cyclic AMP generation following stimulation of HEK.β_2AR cells with isoprenaline (1 μM).

Table 1 Radioligand binding assays

Cell type	*Lovastatin* (10 μM)	*[^{3}H]-Iloprost bound* (fmol mg^{-1} protein)[a]
HEK.mIP	–	3160 ± 380
	+	2970 ± 490
HEK.mIP^{SSLC}	–	3573 ± 510
	+	3672 ± 600
HEK.hIP	–	1580 ± 160
	+	1444 ± 70
HEK 293	–	12.0 ± 2.4
	+	12.5 ± 0.9
HEL	–	44.3 ± 2.6
	+	41.5 ± 4.4
Cell type	*Lovastatin* (10 μM)	*[^{3}H]-CGP-12177 bound* (fmol mg^{-1} protein)[b]
HEK.β_2 AR	–	95.1 ± 5.0
	+	99.7 ± 7.3
HEK 293	–	14.9 ± 3.5
	+	13.4 ± 5.0

[a]Radioligand binding assays were carried out on membrane fractions in the presence of 4 nM [^{3}H]-iloprost. [b]Radioligand binding assays were carried out on whole cells in the presence of 25 nM [^{3}H]-CGP-12177. Data are presented as the mean ± s.e.mean ($n=4$).

Time dependent effects of the statins on IP receptor-mediated cyclic AMP generation

In HEK.mIP cells both statins significantly reduced cicaprost mediated cyclic AMP generation following 8 h incubation such that following 24 h incubation, the level of cicaprost mediated cyclic AMP generation was reduced to 13.7 and 12.5% in the presence of lovastatin and cerivastatin, respectively, relative to control, non-treated cells (Figure 2A,B). In HEL cells, both statins significantly reduced IP receptor signalling following 8 h incubation such that following 24 h incubation, cicaprost mediated cyclic AMP generation was reduced to 51.4 and 44.3% in the presence of lovastatin and cerivastatin, respectively (Figure 2A,B). There

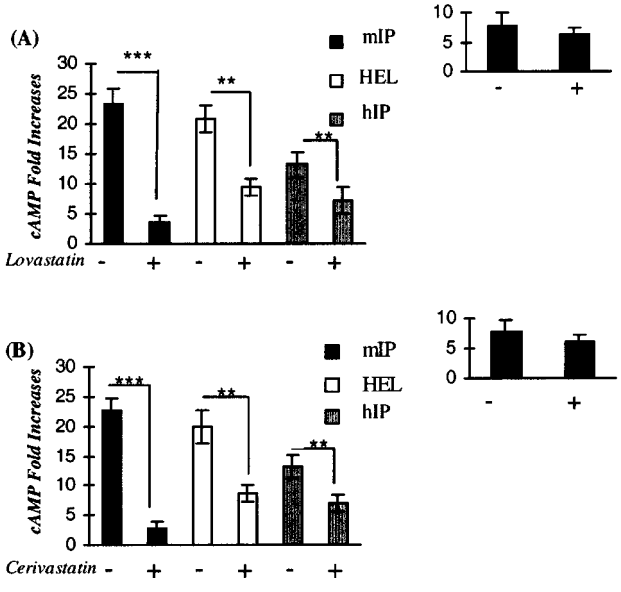

Figure 1 Effect of lovastatin and cerivastatin on IP receptor-mediated cyclic AMP generation. HEK.mIP cells (mIP), HEL cells (HEL), HEK.hIP cells (hIP), or HEK.β_2AR cells (β_2AR; inset to panels) were preincubated with (+) or without (–) 10 μM lovastatin (A) or with (+) and without (–) 0.5 μM cerivastatin (B) for 16 h prior to harvesting. Cells were stimulated with 1 μM cicaprost or, in the case of the β_2AR, with 10 μM alprenolol. The asterisks (*) indicate that cicaprost mediated cyclic AMP generation was significantly reduced in the presence of the statins compared to the corresponding control cells, where ** indicates $P<0.01$ and *** indicates $P<0.001$.

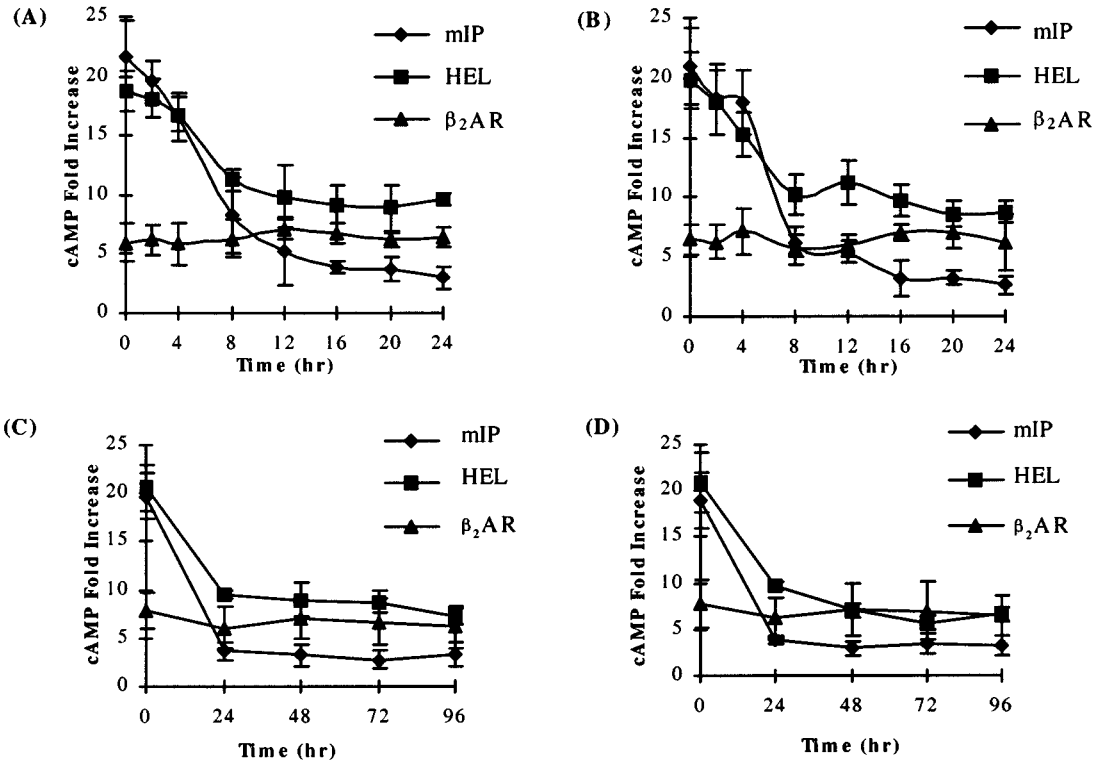

Figure 2 Time dependent effect of lovastatin and cerivastatin on IP receptor-mediated cyclic AMP generation. HEK.mIP cells (mIP), HEL cells (HEL) or HEK.β_2 AR cells (β_2AR) were preincubated with 10 μM lovastatin (A) or 0.5 μM cerivastatin (B) for 0–24 h. Alternatively, cells were exposed to repeat doses of 10 μM lovastatin (C) or 0.5 μM cerivastatin (D) every 24 h for up to 96 h prior to harvesting. Cells were stimulated with 1 μM cicaprost or, in the case of the β_2AR, with 10 μM alprenolol.

were no statistical differences in the overall level of inhibition induced by 10 μM lovastatin compared with 0.5 μM cerivastatin in either HEK.mIP cells or HEL cells; however, the extent of inhibition of cyclic AMP generation by the mIP receptor expressed in HEK.mIP cells was significantly greater than that of the hIP receptor expressed in HEL cells to either lovastatin ($P<0.0002$, 24 h) or cerivastatin ($P<0.0005$, 24 h).

To establish whether repeat exposure to the statins could cause further reductions in IP receptor signalling, fresh aliquots of lovastatin or cerivastatin were added to the cells every 24 h for up to 96 h (Figure 2C,D). No further reductions in IP receptor mediated cyclic AMP generation were observed following repeat exposure of HEK.mIP or HEL cells to lovastatin or cerivastatin for 24, 48, 72 or 96 h. However, the extent of inhibition of cyclic AMP generation by mIP receptor was significantly greater than that of the hIP receptor expressed in HEL cells to lovastatin ($P<0.038$ at 96 h) or cerivastatin ($P<0.05$ at 96 h) treatment. Neither statin affected signalling by the β_2AR even following prolonged incubation (24 h) with either lovastatin ($P>0.84$) or cerivastatin ($P>0.91$; Figure 2A,B) or following repeat exposure to either statin (Figure 2C,D).

Concentration dependent effects of the statins on IP receptor-mediated cyclic AMP generation

From concentration response studies (Figure 3), IC_{50} values for lovastatin and cerivastatin in HEK.mIP and HEL cells were determined (Table 2). Whereas these IC_{50} values indicated that the hIP receptor was significantly more sensitive than the mIP receptor to lovastatin ($P<0.0004$) or cerivastatin ($P<0.013$), the extent of inhibition of cyclic AMP generation by the mIP receptor was significantly greater than that of the hIP receptor to either statin treatment, even at the highest concentrations used. Signalling in HEK.mIP cells was reduced to 16.5 and 15% following lovastatin (20 μM) and cerivastatin (5 μM; data not shown) treatment, respectively, whereas signalling in HEL cells was reduced to 40.3 and 29% following lovastatin (20 μM) and cerivastatin (5 μM; data not shown) treatment, respectively. In each case, the statins had no effect on alprenolol mediated signalling by HEK.β_2AR cells (Figure 3D,H).

To exclude the possibility that the apparent differential extent of inhibition of cyclic AMP generation by the mIP receptor, expressed in HEK 293 cells, to that of the hIP receptor, endogenously expressed in HEL cells, to statin treatment may be due to unexplained differences between the host cells, we extended our studies to establish a stable HEK 293 cell line (HEK.hIP cells) over-expressing the hIP receptor. Preincubation of HEK.hIP cells with lovastatin (Table 1; $P>0.46$) or cerivastatin (0.5 μM; $P>0.19$; data not shown) had no significant effect on [^{3}H]-iloprost binding.

Pretreatment of HEK.hIP cells with lovastatin (10 μM) or cerivastatin (0.5 μM) for 16 h led to 50.7 and 46.9% reductions in cicaprost mediated cyclic AMP generation, respectively (Figure 1). From the concentration response data, IC_{50} values of 0.8 μM lovastatin (Figure 3C, Table 2) and 60 nM cerivastatin (Figure 3G, Table 2) in HEK.hIP cells were determined. These IC_{50} values were consistent with those previously found in HEL cells but which differed

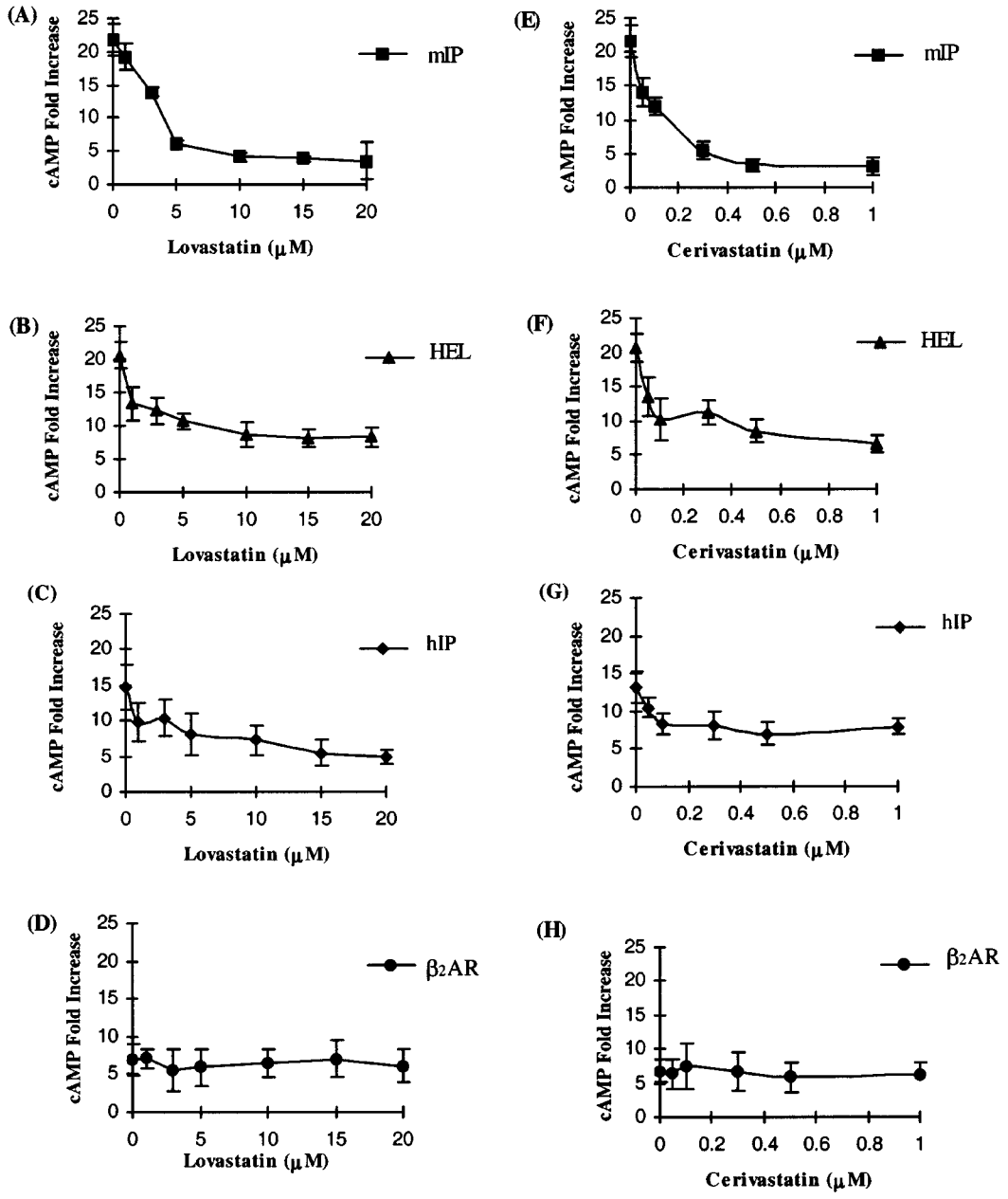

Figure 3 Concentration dependent effects of lovastatin and cerivastatin on IP receptor-mediated cyclic AMP generation. HEK.mIP cells (mIP; A and E), HEL cells (HEL; B and F), HEK.hIP cells (hIP; C and G), or HEK.β_2 AR cells (β_2AR; D and H) were preincubated with 0–20 μM lovastatin (A–D) or 0–1 μM cerivastatin (E–H) for 16 h prior to harvesting. Cells were stimulated with 1 μM cicaprost (A–C, E–G) or, in the case of the β_2AR (D and H), with 10 μM alprenolol.

significantly from those in HEK.mIP cells (Table 2). Additionally, consistent with previous observations, the extent of inhibition of cyclic AMP generation by the mIP receptor expressed in HEK.mIP cells was significantly greater than that of the hIP receptor expressed in HEK.hIP cells to lovastatin ($P<0.0001$) and cerivastatin ($P<0.0001$).

The effect of the statins on IP-receptor mediated $[Ca^{2+}]_i$ mobilization

We extended our studies to investigate the effect of the statins on cicaprost mediated $[Ca^{2+}]_i$ mobilization by the mIP and hIP receptor or, as controls for a non-isoprenylated receptor, in response to stimulation of HEK.TPα10 cells, stably over-expressing the α isoform of the human prostanoid TP receptor (TP), with the selective TP agonist U46619. Stimulation of HEK.mIP cells, HEL cells and HEK.hIP cells with cicaprost each resulted in an increase in $[Ca^{2+}]_i$ mobilization (Table 3). Cicaprost mediated $[Ca^{2+}]_i$ mobilization by HEK.mIP cells was reduced to 66.8 and 22.4% in lovastatin (10 μM; 16 h) and cerivastatin (0.5 μM; 16 h) treated cells, respectively (Table 3). From concentration response studies, IC_{50} values of 4.2 μM lovastatin (Figure 4A and Table 2) and 90 nM

cerivastatin (Figure 4D and Table 2) were determined in HEK.mIP cells.

In HEL cells, cicaprost mediated $[Ca^{2+}]_i$ mobilization was reduced to 44.9 and 23.3% in lovastatin (10 μM; 16 h) and cerivastatin (0.5 μM; 16 h) treated cells, respectively (Table 3). From concentration response studies, respective IC_{50} values of 3.1 μM lovastatin (Figure 4B and Table 2) and 65 nM cerivastatin (Figure 4E and Table 2) were determined in HEL cells. Stimulation of HEK.hIP cells with cicaprost resulted in an increase in $[Ca^{2+}]_i$ mobilization which was reduced to 41.9% in lovastatin (10 μM) treated cells, and to 45.3% in cerivastatin (0.5 μM) treated cells (Table 3). From the concentration response curves (Figure 4C,F), IC_{50} values of lovastatin and cerivastatin in HEK.hIP were 2.6 μM and 63 nM, respectively (Table 2). Thus, the statins significantly impaired IP receptor mediated $[Ca^{2+}]_i$ mobilization in HEL cells, in HEK.mIP cells and in HEK.hIP cells with each cell type being more sensitive to cerivastatin than lovastatin treatment. In contrast, U46619 mediated $[Ca^{2+}]_i$ mobilization in HEK.TPα10 cells, transiently co-transfected with $G\alpha_q$, (Table 3) was not affected by pre-exposure of cells to either lovastatin (Table 3; $P>0.4$) or cerivastatin (Table 3; $P>0.41$).

Table 2 IC_{50} values for lovastatin and cerivastatin

	Cyclic AMP [a] (IC_{50})		*$[Ca^{2+}]_i$* [b] (IC_{50})	
Cell type	*Lovastatin* (μM)	*Cerivastatin* (nM)	*Lovastatin* (μM)	*Cerivastatin* (nM)
HEK.mIP	3.5	80	4.2	90
HEL	0.85	50	3.1	65
HEK.hIP	0.8	60	2.6	63

Cells were incubated with 0–20 μM lovastatin or 0–5 μM cerivastatin for 16 h. Thereafter, IP receptor mediated cyclic AMP[a] and intracellular $[Ca^{2+}]_i$ mobilization[b] following stimulation of cells with 1 μM cicaprost was evaluated. Inhibitory concentration 50 (IC_{50}) values for lovastatin (μM) and cerivastatin (nM) were determined and are presented.

Table 3 Effect of the statins on IP- and TP-receptor mediated $[Ca^{2+}]_i$ mobilization

	Growth condition		
Cell type	*Control* $\Delta[Ca^{2+}]_i$ (nM)	*Lovastatin* $\Delta[Ca^{2+}]_i$ (nM)	*Cerivastatin* $\Delta[Ca^{2+}]_i$ (nM)
HEK.mIP[a]	88.0±3.85	58.0±1.53**	19.7±0.61***
HEL[a]	81.1±4.25	36.4±3.02**	18.9±1.9***
HEK.hIP[a]	139±6.36	58.2±3.61***	63.0±1.56**
HEK.TPα10+$G\alpha_q$[b]	144±18.2	116±19.9	115±16.1

Cells were incubated without (Control) or with 10 μM lovastatin (Lovastatin) or with 0.5 μM cerivastatin (Cerivastatin) for 16 h. Thereafter, cells were stimulated with 1 μM cicaprost[a] or with 1μM U46619[b] and agonist mediated changes in intracellular calcium mobilization ($\Delta[Ca^{2+}]_i$ (nM)) were determined. The asterisks indicate that cicaprost mediated intracellular $[Ca^{2+}]_i$ mobilization was significantly reduced in the presence of lovastatin or cerivastatin compared to the corresponding control cells, where ** indicates $P<0.01$ and *** indicates $P<0.001$.

The effect of the statins on IP receptor-mediated desensitization of TP signalling

We have recently established that the TPα, but not the TPβ, isoform of the human TXA_2 receptor (TP) is subject to direct IP receptor-mediated desensitization (Walsh *et al*., 2000b). Thus, in view of their inhibitory effects on IP receptor signalling, we sought to investigate the effect of the statins on IP receptor-mediated desensitization or counter-regulation of TP receptor responses. Both HEK.TPα10 cells and HEK.TPβ3 cells showed efficient U46619 mediated $[Ca^{2+}]_i$ mobilization which was not significantly affected by statin treatment (Figure 5 and Table 4). Whereas stimulation of HEK.TPα10 cells or HEK.TPβ3 cells with cicaprost failed to mediate a measurable rise in $[Ca^{2+}]_i$ mobilization (Figure 5), initial stimulation of HEK.TPα10 cells with cicaprost reduced U46619 mediated signalling to 38% ($P<0.008$; Figure 5A and Table 4); in contrast, U46619 mediated signalling by HEK.TPβ3 cells was unaffected by cicaprost ($P>0.82$; Figure 5B and Table 4). Initial stimulation of lovastatin treated cells with cicaprost reduced U46619 mediated signalling by HEK.TPα10 cells to 54% ($P<0.05$; Figure 5C and Table 4); however the lovastatin treated cells were significantly less sensitive ($P<0.029$; Table 4) to cicaprost desensitization than were HEK.TPα10 cells not treated with lovastatin. In contrast, initial stimulation of cerivastatin treated cells with cicaprost did not affect U46619 mediated signalling by HEK.TPα10 cells ($P>0.137$; Figure 5E and Table 4) and thus, cerivastatin treated cells were significantly less sensitive ($P<0.0038$; Table 4) to cicaprost desensitization than were those HEK.TPα10 cells not treated with statin. At lower concentrations, lovastatin (1 μM) or cerivastatin (50 nM) did not affect cicaprost mediated desensitization of TPα signalling in HEK.TPα10 cells (data not shown). Consistent with previous data, U46619 mediated signalling by HEK.TPβ3 cells was unaffected by cicaprost (Table 4; $P>0.3-0.68$) irrespective of pre-treatment of cells with or without statin (Figure 5B,D,F; Table 4).

Discussion

In a recent study we established that the IP receptor may be unique among GPCRs in that it is isoprenylated by a C15 farnesyl moiety at a cysteine residue within its C-tail region (Hayes *et al*., 1999). Through site directed mutagenesis we established that whereas isoprenylation was not required for ligand binding by mIP receptor, it was absolutely required for its efficient coupling to the effectors adenylyl cyclase and PLC and, hence, for signalling by the IP receptor. In provisional studies, inhibition of isoprenylation in the presence of the HMG CoA reductase inhibitor lovastatin significantly reduced IP receptor mediated cyclic AMP generation (Hayes *et al*., 1999). The statins as competitive inhibitors of HMG CoA reductase, the rate limiting enzyme of cholesterol biosynthesis, can be used to interfere with protein isoprenylation and therefore, represent important research tools in elucidating the functional role of protein isoprenylation (Maltese, 1990). In the clinical setting, the statins are widely and effectively used in the treatment of hypercholesterolaemia, a major risk factor in the development of cardiovascular disease, in particular atherosclerosis (Farnier & Davignon,

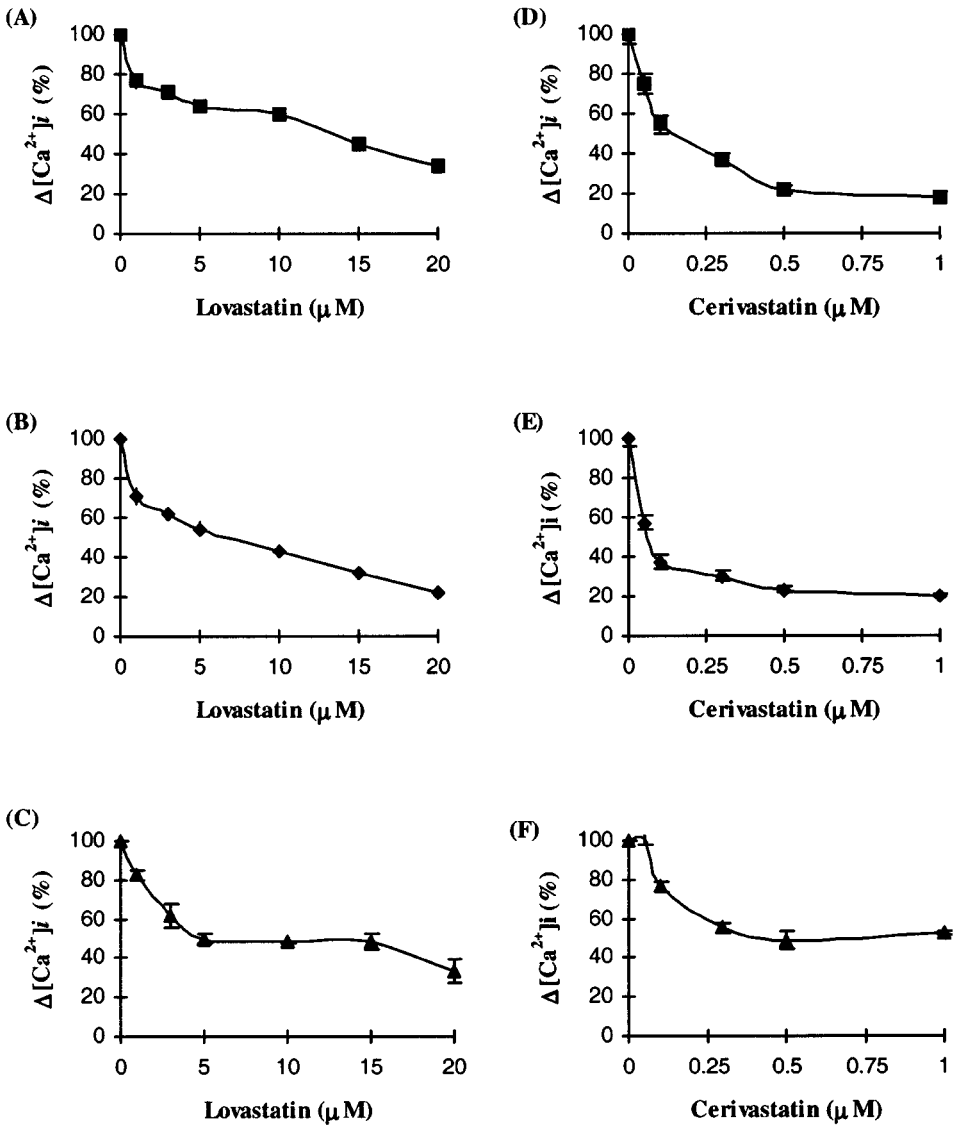

Figure 4 Concentration dependent effects of lovastatin and cerivastatin on IP receptor-mediated $[Ca^{2+}]_i$ mobilization. HEK.mIP cells (A and D), HEL cells (B and E) and HEK.hIP cells (C and F), were preincubated with 0–20 μM lovastatin (A–C) or 0–1 μM cerivastatin (D–F) for 16 h prior to harvesting. Data were calculated as changes in intracellular Ca^{2+} mobilized ($\Delta[Ca^{2+}]_i \pm$ s.e.mean, nM) and are expressed as a percentage (%) relative to control, non-statin treated cells ($\Delta[Ca^{2+}]_i \pm$ s.e.mean, %).

1998). Thus, in view of the central role of the IP receptor within the cardiovascular system coupled to our finding for a functional requirement for isoprenylation of the IP receptor, we sought to investigate in detail the effects of lovastatin and cerivastatin on signalling by the IP receptor in whole cells. Lovastatin serves as a reference compound and clinically is known to be effective only at milligram concentrations (Alberts *et al*., 1980; Hanefeld *et al*., 1999). On the other hand cerivastatin, a fully synthetic pure enantiomer, has been shown to be therapeutically active at microgram concentrations (Stein *et al*., 1997; 1999; Hanefeld *et al*., 1999).

Thus, in the present study we investigated the effects of lovastatin and cerivastatin on signalling by the mIP receptor, stably over-expressed in HEK.mIP cells and by the hIP receptor, endogenously expressed in HEL cells. To further investigate the differential sensitivity of the hIP receptor versus the mIP receptor to the statins, the effects of lovastatin and cerivastatin on signalling by the hIP receptor stably over-expressed in HEK.hIP cells was also examined. Throughout these studies, the effects of the statins on IP receptor signalling were compared to signalling by the non-isoprenylated β_2 AR or by TPα receptor. Initially, in view of the established relative potencies of the statins under study, the effect of 10 μM lovastatin and 0.5 μM cerivastatin on IP receptor mediated cyclic AMP generation was investigated. In HEK.mIP cells, both statins reduced mIP receptor signalling to 10–15% whereas in HEL and in HEK.hIP cells, hIP receptor signalling was reduced to approximately 45% by either statin. No significant decreases in β_2 AR signalling were observed following exposure of HEK.β_2AR cells to either statin, confirming that the observed effects of the statins are targeted to the IP receptor itself and not to some other component of the signalling system. Lovastatin or cerivastatin pretreatment had no significant effect on ligand

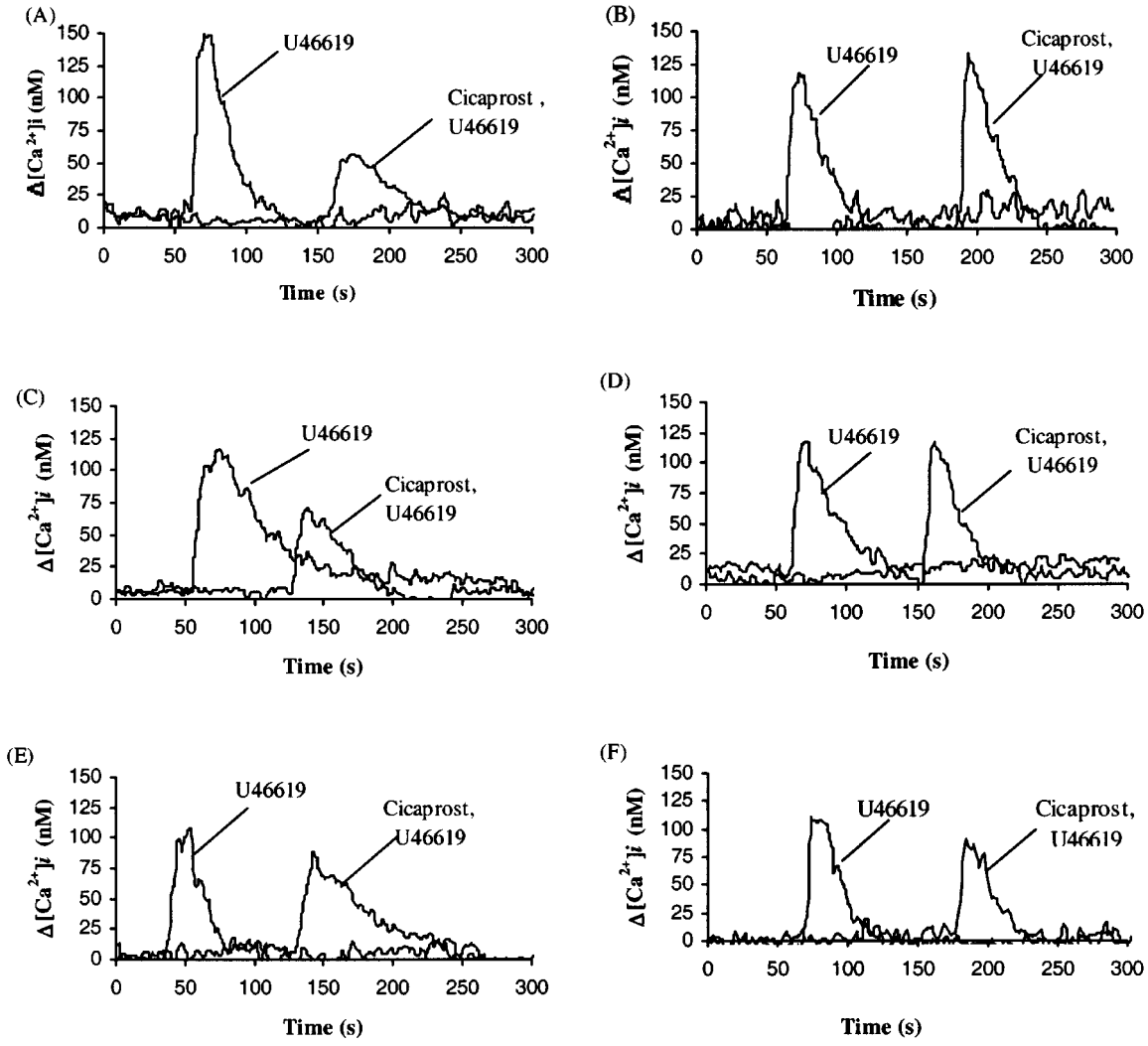

Figure 5 Effect of lovastatin and cerivastatin on IP receptor-mediated desensitization of TP receptor signalling. HEK.TPα10 cells (A,C and E) and HEK.TPβ3 cells (B,D and F), transiently co-transfected with $G\alpha_q$, were preincubated with vehicle (A and B), 10 μM lovastatin (C and D) or 0.5 μM cerivastatin (E and F) for 16 h prior to harvesting. Cells were stimulated with 1 μM U46619 alone at 30–50 s (U46619) or 1 μM cicaprost, at 30–50 s, followed by 1 μM U46619, at 125–175 s (Cicaprost, U46619).

binding by IP receptors expressed in HEL or HEK 293 cells or by the control non-isoprenylated β_2 AR, excluding the possibility that the effects of the statins on IP receptor signalling may be due to decreases in overall levels of IP receptor expression. This was consistent with previous data whereby disruption of IP receptor isoprenylation by site directed mutagenesis had no significant effect on its ligand binding properties (Hayes *et al*., 1999).

Whereas both lovastatin and cerivastatin exhibited significant time dependent reductions in mIP and hIP receptor signalling, the extent of inhibition of cyclic AMP generation by the mIP receptor was greater than that of the hIP receptor to either statin even following their repeat administration over a period of 96 h. Whereas IC_{50} values indicated that the hIP receptor was significantly more sensitive than the mIP receptor to lovastatin ($P<0.0004$) and cerivastatin ($P<0.039$), concentration response studies also indicated that the extent of inhibition of cyclic AMP generation by the mIP receptor was significantly greater than that of the hIP receptor to either statin, even at the highest concentrations used. In contrast, signalling by the β_2 AR was unaffected by either statin, irrespective of the concentration.

We next sought to investigate the effects of the statins on IP receptor mediated $[Ca^{2+}]_i$ mobilization. Initially we examined the relative sensitivities of cicaprost mediated $[Ca^{2+}]_i$ mobilization by the mIP and hIP receptor to lovastatin and cerivastatin and compared these effects to those of the statins on U46619 mediated $[Ca^{2+}]_i$ mobilization by the non-isoprenylated TPα isoform. Consistent with previous reports (Hayes *et al*., 1999; Van der Vuurst *et al*., 1997), the mIP and hIP receptors exhibited efficient mobilization of $[Ca^{2+}]_i$ in response to cicaprost, confirming that IP receptors from both species couple to PLC activation. Stimulation of HEK.TPα10, HEK.TPβ3 or HEK 293 (Hayes *et al*., 1999) cells with cicaprost failed to exhibit a rise in $[Ca^{2+}]_i$ mobilization indicating that the level of endogenous IP receptor expressed in HEK 293 cells (Table 1) may not be sufficient to support a measurable rise in $[Ca^{2+}]_i$. These data also confirm that the cicaprost mediated $[Ca^{2+}]_i$ response observed in HEK.mIP and HEK.hIP cells was mediated

Table 4 The effect of the statins on IP receptor mediated desensitization of TP receptor signalling

Changes in U46619 mediated $[Ca^{2+}]_i$ mobilization ($\Delta[Ca^{2+}]_i \pm$s.e.mean, nM; $n=4$)			
		Treatment	
HEK.TPα10 cells + $G\alpha_q$ Stimulating agent	*− Statin* (nM)	*+ Lovastatin* (nM)	*+ Cerivastatin* (nM)
U46619	144 ± 18.1	116 ± 17.8	115 ± 17.7
Cicaprost, U46619	55.0 ± 3.03	65.9 ± 1.93*	81.5 ± 3.43**
HEK.TPβ3 cells + $G\alpha_q$ Stimulating agent			
U46619	125 ± 19.1	115 ± 16.5	127 ± 22.2
Cicaprost, U46619	118 ± 19.3	128 ± 11.5	102 ± 16.2

HEK.TPα10 or HEK.TPβ3 cells, transiently co-transfected with $G\alpha_q$, were pre-incubated for 16 h in the absence or presence of lovastatin (10 μM) or cerivastatin (0.5 μM) and then stimulated with U46619 (1 μM) alone or with cicaprost (1 μM) followed by U46619 (1 μM). U46619 mediated Ca^{2+} mobilizations were represented as mean changes $\Delta[Ca^{2+}]_i \pm$s.e.mean, nM; $n=4$. The asterisks indicate that cicaprost mediated desensitization of U46619 mediated intracellular $[Ca^{2+}]_i$ mobilization was significantly reduced in the presence of lovastatin or cerivastatin compared to the corresponding control cells, where * indicates $P<0.05$ and ** indicates $P<0.01$.

through IP receptors and not through other unrelated prostanoid receptors. Both lovastatin and cerivastatin exhibited time (data not shown) and concentration dependent inhibitions of cicaprost mediated $[Ca^{2+}]_i$ mobilization. From concentration response studies, IC_{50} values indicated that the hIP receptor is more sensitive to statin treatment than the mIP receptor. Thus, taken together these studies investigating the effects of the statins on IP receptor mediated cyclic AMP generation and $[Ca^{2+}]_i$ mobilization imply that there are differences in the structure/function properties and the influence of isoprenylation on the signalling behaviour of the mIP and hIP receptors. In essence, these structure/function differences indicate that the extent of inhibition of cyclic AMP generation due to statin treatment by the mIP receptor is significantly greater than that of the hIP receptor whereas the IC_{50} data confirm that the hIP receptor is more sensitive than the mIP receptor to statin treatment.

Differences in the signalling behaviour of the mIP and hIP receptors have been previously alluded to in the scientific literature (Smyth *et al*., 1998; Hayes *et al*., 1999). In studies investigating the functional requirement for protein isoprenylation of the mIP receptor, we have previously established that, through site directed mutagenesis of the critical cysteine residue within the 'CAAX' motif of mIP, leading to generation of mIP^{SSLC}, cicaprost mediated cyclic AMP generation by mIP^{SSLC} was almost completely abolished whereas cicaprost mediated $[Ca^{2+}]_i$ mobilization was significantly reduced in a concentration dependent manner but was not abolished (Hayes *et al*., 1999). On the other hand, Smyth *et al*. (1998) reported that deletion of a substantial portion of the C-tail of the hIP receptor including deletion of the 'CAAX' motif, generated a receptor that exhibited identical coupling to adenylyl cyclase relative to the wild type hIP receptor, but which exhibited substantially diminished ability to couple to PLC activation. A more complete understanding of the species specific behaviour of the mouse and human IP receptors will require detailed structure/function analyses of the individual receptors, in particular within their unique C-tail regions.

In studies investigating the intermolecular cross talk between IP and TP receptor signalling, we have recently established that the α, but not the β isoform, of the h.TP receptor is subject to counter regulation or desensitization by the IP receptor, mediated through direct protein kinase A dependent phosphorylation of Ser^{329} within the unique C-tail of TPα (Walsh *et al*., 2000b). In view of the inhibitory effect of the statins on IP receptor signalling, we sought to establish whether the statins may interfere in IP receptor mediated desensitization or counter regulation of TP receptor responses. Consistent with previous studies (Walsh *et al*., 2000b), both HEK.TPα10 and HEK.TPβ3 cells exhibited efficient $[Ca^{2+}]_i$ mobilization in response to U46619 which was not significantly affected by pretreatment of either cell type with either statin. Whereas stimulation of HEK.TPα10 or HEK.TPβ3 cells with cicaprost did not mediate significant or measurable rises in $[Ca^{2+}]_i$ mobilization *per se*, initial stimulation of cells with cicaprost significantly desensitized signalling by the TPα, but not the TPβ, receptor. In lovastatin treated cells, cicaprost significantly reduced $[Ca^{2+}]_i$ mobilization by TPα, but not TPβ; however, the lovastatin treated HEK.α10 cells were significantly less sensitive ($P<0.029$) to cicaprost desensitization than were non-statin treated cells. In cerivastatin treated cells, cicaprost failed to desensitize $[Ca^{2+}]_i$ mobilization by the TPα receptor in response to U46619; thus, cerivastatin significantly ($P<0.0038$) impaired IP receptor mediated desensitization of TPα responses. On the other hand, cerivastatin had no affect on signalling by the TPβ isoform.

Taken together, we have demonstrated that both lovastatin and cerivastatin inhibit signalling by the mIP and hIP receptor in whole cells in a time and concentration dependent manner. In the case of cerivastatin, it was noteworthy that the IC_{50} values for inhibition of IP receptor mediated cyclic AMP generation and $[Ca^{2+}]_i$ mobilization were 50–90 nM cerivastatin, concentrations which are reflective of the maximum plasma concentration of 28.7 nM (13.8 μg L^{-1}) and the 24 h area under the curve (or AUC_{0-24}) of 145 nM (69.9 μg L^{-1}) achieved *in vivo* in humans for cerivastatin following daily dosing of 0.8 mg for 28 days (Stein *et al*., 1999). The physiological effects of statin treatment *in vivo* on IP receptor signalling, IP receptor function and counter-regulation of TP receptor signalling and control of vascular haemostasis is at present unknown. Such physiological effects may be particularly pronounced under conditions where elevated blood concentrations of statins occur, in particular for potent stains such as cerivastatin. Drugs which are either inhibitors or substrates for cytochrome P450–3A4 isotype, such as cyclosporine A, erythromycin, clarithromycin, nefazodone, protease inhibitors (Maronm *et al*., 2000) have been shown to inhibit the metabolism and bio-transformation of the statins *in vivo*, leading to elevated statin blood concentrations (Corsini *et al*., 1999; Hanefeld *et al*., 1999). This, in turn, increases the chances of adverse effects associated with statin treatment, in particular liver toxicity and muscle toxicity or myopathy (defined as muscle pain or weakness). A 3–5 fold increase in plasma levels of cerivastatin have been observed in patients receiving

cyclosporine A (Mück, 1998); similar increases in blood levels have been observed for other statins including pravastatin and fluvastatin. Co-administration of the protease inhibitors ritonavir and saquinavir with simvastatin and atorvastatin led to increased statin plasma concentrations of 31.6 fold and 4.5 fold, respectively (Fichtenbaum *et al*., 2000). Other adverse effects associated with statin therapy or following drug-drug interactions *in vivo* include back pain, headache and acute renal failure (Hanefeld *et al*., 1999). What correlation, if any, exists between statin therapy and associated adverse effects due to interference in IP receptor isoprenylation and IP receptor signalling remains to be determined. However, given our findings that concentrations as low as 50 nM cerivastatin can interfere with IP receptor function, it is possible that clinical settings where elevated levels of statins, particularly cerivastatin, occur could interfere in IP receptor signalling and indeed with IP receptor-regulated vascular haemostasis. It is also possible that other unexplained secondary pleiotropic effects of statin treatment which are not related to their lipid lowering properties, such as their documented effects on endothelial function, inflammatory responses, plaque stability, thrombus formation, decreased smooth muscle cell migration and proliferation (Corsini *et al*., 1999) may in part be attributed to interference in IP receptor isoprenylation and function.

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References

ALBERTS, A.W., CHEN, J., KURON, G., HUNT, V., HUFF, J., HOFFMAN, C., ROTHROCK, J., LOPEZ, M., JOSHUA, H., HARRIS, E., PATCHETT, A., MONAGHAN, R., CURRIE, S., STAPLEY, E., ALBERS-SCHONBERG, G., HENSENS, D., HIRSCHFIELD, J., HOOGSTEEN, K., LIESCH, J. & SPRINGER, J. (1980). Mevinolin: a highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol-lowering agent. *Proc. Natl. Acad. Sci. U.S.A.,* **77,** 3957–3961.

BOIE, Y., RUSHMORE, T.H., DARMON-GOODWIN, A., GRYGORCZYK, R., SLIPETZ, D.M., METTERS, K.M. & ABRAMOVITZ, M. (1994). Cloning and expression of a cDNA for the human prostanoid IP receptor. *J. Biol. Chem.,* **269,** 12173–12178.

CHATZIPANTELI, K., RUDOLPH, S. & AXELROD, L. (1992). Coordinate control of lipolysis by prostaglandin E2 and prostacyclin in rat adipose tissue. *Diabetes,* **41,** 927–935.

COLEMAN, R.A., SMITH, W.L. & NARUMIYA, S. (1994). International Union of Pharmacology classification of prostanoid receptors: properties, distribution, and structure of the receptors and their subtypes. *Pharmacol. Rev.,* **46,** 205–229.

CORSINI, A., BELLOSTA, S., BAETTA, R., FUMAGALLI, R., PAOLETTI, R. & BERNINI, F. (1999). New insights into the pharmacodynamic and pharmacokinetic properties of statins. *Pharmacol. & Ther.,* **84,** 413–428.

FARNIER, M. & DAVIGNON, J. (1998). Current and future treatment of hyperlipidemia: the role of statins. *Am. J. Cardiol.,* **82,** 3J–10J.

FICHTENBAUM, C., GERBER, S.J., ROSENKRANZ, S., SEGAL, Y., BLASCHKE, T., ABERG, J., ROYAL, M., BURNING, W., LAMB, K., FERGUSON, E., ALSTON, B. & AWEEKA, F. (2000). Pharmacokinetic interactions between protease inhibitors and selected HMG-CoA reductase inhibitors. Abstract LB6. Published abstract presented at the *7th Conference on Retroviruses and Opportunistic Infections*, San Francisco, February 2000.

GAGNON, A.W., KALLAL, L. & BENOVIC, J.L. (1998). Role of clathrin-mediated endocytosis in agonist-induced down regulation of the β_2 adrenergic receptor. *J. Biol. Chem.,* **273,** 6976–6981.

GUIJARRO, C., BLANCO-COLIO, L.M., ORTEGO, M., ALONSO, C., ORTIZ, A., PLAZA, J.J., DIAZ, C., HERNANDEZ, G. & EGIDO, J. (1998). 3-Hydroxy-3-methylglutaryl Coenzyme A reductase and isoprenylation inhibitors induce apoptosis of vascular smooth muscle cells in culture. *Circ. Res.,* **83,** 490–500.

HANEFELD, M., DESLYPERE, J.P., OSE, L., DURRINGTON, P.N., FARNIER, M. & SCHMAGE, N. (1999). Efficacy and safety of 300 micrograms and 400 micrograms cerivastatin once daily in patients with primary hypercholesterolaemia: a multicentre, randomized, double-blind, placebo-controlled study. *J. Int. Med. Res.,* **27,** 115–129.

HAYES, J.S., LAWLER, O.A., WALSH, M.-T. & KINSELLA, B.T. (1999). The prostacyclin receptor is isoprenylated. Isoprenylation is required for efficient receptor-effector coupling. *J. Biol. Chem.,* **274,** 23707–23718.

KINSELLA, B.T., O'MAHONY, D.J. & FITZGERALD, G.A. (1997). The human thromboxane A_2 receptor alpha isoform (TPalpha) functionally couples to the G proteins G_q and G_{11} *in vivo* and is activated by the isoprostane 8-epi prostaglandin F2 alpha. *J. Pharmacol. Exp. Ther.,* **281,** 957–964.

MACMAHON, S., SHARPE, N., GAMBLE, G., HART, H., SCOTT, J., SIMES, J. & WHITE, H. (1998). Effects of lowering average or below-average cholesterol levels on the progression of carotid atherosclerosis – results of the LIPID-atherosclerosis substudy. *Circulation,* **97,** 1784–1790.

MALTESE, W.A. (1990). Posttranslational modification of proteins by isoprenoids in mammalian cells. *FASEB J.,* **4,** 3319–3328.

MARONM, D.J., FAZIO, S. & LINTON, M.F. (2000). Current perspectives on statins. *Circulation,* **101,** 207–213.

MÜCK, W. (1998). Rational assessment of the interaction profile of cerivastatin supports its low propensity for drug interactions. *Drugs,* **56** (Suppl 1), 15–23; discussion 33.

NAMBA, T., OIDA, H., SUGIMOTO, Y., KAKIZUKA, A., NEGISHI, M., ICHIKAWA, A. & NARUMIYA, S. (1994). cDNA cloning of a mouse prostacyclin receptor. Multiple signalling pathways and expression in thymic medulla. *J. Biol. Chem.,* **269,** 9986–9992.

NARUMIYA, S., SUGIMOTO, Y. & USHIKUBI, F. (1999). Prostanoid receptors: Structure, Properties and Functions. *Physiological Reviews,* **79,** 1193–1226.

NEGISHI, M., SUGIMOTO, Y. & ICHIKAWA, A. (1995). Molecular mechanisms of diverse actions of prostanoid receptors. *Biochim. Biophys. Acta,* **1259,** 109–119.

SACKS, F.M., PFEFFER, M.A., MOYE, L.A., ROULEAU, J.L., RUTHERFORD, J.D., COLE, T.G., BROWN, L., WARNICA, J.W., ARNOLD, J.M.O., WUN, C., DAVIS, B.R. & BRAUNWELD, E. (1996). The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. *N. Engl. J. Med.,* **335,** 1001–1009.

SAKAI, A., YAJIMA, M. & NISHIO, S. (1990). Cytoprotective effect of TRK-100, a prostacyclin analogue, against chemical injuries in cultured human vascular endothelial cells. *Life Sci.,* **47,** 711–719.

SCHWANER, I., OFFERMANNS, S., SPICHER, K., SEIFERT, R. & SCHULTZ, G. (1995). Differential activation of Gi and Gs proteins by E- and I-type prostaglandins in membranes from the human erythroleukaemia cell line, HEL. *Biochim. Biophys. Acta.,* **1265,** 8–14.

SMYTH, E.M., LI, W.H. & FITZGERALD, G.A. (1998). Phosphorylation of the prostacyclin receptor during homologous desensitization. A critical role for protein kinase c. *J. Biol. Chem.,* **273,** 23258 – 23266.

STEIN, E., ISAACSOHN, J., STOLTZ, R., MAZZU, A., LIU, M.C., LANE, C. & HELLER, A.H. (1999). Pharmacodynamics, safety, tolerability, and pharmacokinetics of the 0.8-mg dose of cerivastatin in patients with primary hypercholesterolemia. *Am. J. Cardiol.,* **83,** 1433 – 1436.

STEIN, E., SPRECHER, D., ALLENBY, K.S., TOSIELLO, R.L., WHALEN, E. & RIPA, S.R. (1997). Cerivastatin, a new potent synthetic HMG Co-A reductase inhibitor: effect of 0.2 mg daily in subjects with primary hypercholesterolemia. *J. Cardiovasc. Pharmacol. Ther.,* **2,** 7 – 16.

THOMPSON, G.R. (1998). Introduction. *Am. J. Cardiol.,* **82,** 1J – 2J.

VAUGHAN, C.J., GOTTO, JR., A.M. & BASSON, C.T. (2000). The evolving role of statins in the management of atherosclerosis. *J. Am. Coll. Cardiol.,* **35,** 1 – 10.

VAN DER VUURST, H., VAN WILLIGEN, G., VAN SPRONSEN, A., HENDRIKS, M., DONATH, J. & AKKERMAN, J.W. (1997). Signal transduction through trimeric G proteins in megakaryoblastic cell lines. *Arterioscler. Thromb. Vasc. Biol.,* **17,** 1830 – 1836.

VANE, J.R. & BOTTING, R.M. (1995). Pharmacodynamic profile of prostacyclin. *Am. J. Cardiol.,* **75,** 3A – 10A.

WALSH, M.-T., FOLEY, J.F. & KINSELLA, B.T. (1998). Characterization of the role of N-linked glycosylation on the cell signalling and expression of the human thromboxane A_2 receptor alpha and beta isoforms. *J. Pharmacol. Exp. Ther.,* **286,** 1026 – 1036.

WALSH, M.-T., FOLEY, J.F. & KINSELLA, B.T. (2000a). Investigation of the role of the carboxyl terminal tails of the α and β isoforms of the human thromboxane A_2 receptor (TP) in mediating receptor: effector coupling. *Biochim. Biophys. Acta.,* **1496,** 164 – 182.

WALSH, M.-T., FOLEY, J.F. & KINSELLA, B.T. (2000b). The α, but not the β, isoform of the human thromboxane A_2 receptor (TP) is a target for prostacyclin mediated desensitization. *J. Biol. Chem.,* **275,** 20412 – 20423.

ZHANG, F.L. & CASEY, P.J. (1996). Protein prenylation: molecular mechanisms and functional consequences. *Annu. Rev. Biochem.,* **65,** 241 – 269.

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