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Statins stimulate arachidonic acid release and prostaglandin I₂ production in rat liver cells

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Abstract

Statins inhibit 3-hydroxy-3-methylglutaryl (HMG-CoA) reductase, the rate limiting step in cholesterol synthesis. They are, therefore, used clinically to lower cholesterol and prevent atherosclerosis. Statins have beneficial effects on multiple organ systems. Some of these effects are found in the absence of significant changes in cholesterol levels. Polyunsaturated fatty acids also inhibit HMG-CoA reductase and have many of the same beneficial effects of statins. Four statins (mevastatin, lovastatin, simvastatin and atorvastatin) have been tested in rat liver cells for their effect on arachidonic acid (AA) release and prostaglandin I₂ production induced in the presence of lactacystin and 12-O-tetradecanoylphorbol-13-acetate. Each statin stimulated release of AA and induced prostaglandin I₂ production. Mevalonate, the product of HMG-CoA reductase, did not reduce the stimulation observed in the presence of simvastatin indicating that HMG-CoA reductase activity is not involved. In view of the multiple biologic properties of AA, the AA released as a result of the action of the statins may play a role in some of the pharmacological effects attributed to these drugs.

Background

The statins, by inhibiting HMG-CoA reductase activity, reduce cholesterol and isoprenoid synthesis [1]. They are being used primarily in the prevention of atherosclerosis [2,3]. They have proven to be beneficial in preventing stroke [4] and may increase bone formation [5] and lower the risk of dementia [6]. Some of the benefits in patients with myocardial infarction are independent of cholesterol levels. Statins also inhibit cellular proliferation and induce apoptosis of tumor cells [7]. Essential fatty acids have several properties similar to the statins [8,9] and Das [10] has suggested that "essential fatty acids and their metabolites may serve as second messengers of the actions of statins."

Statins affect prostaglandin (PG) production. Mevastatin or lovastatin, at 25 μM, induce PGI₂ production and cy-

clooxygenase (COX)-2 in human aorta smooth muscle cells [11]. Mevalonate and geranylgeranyl-pyrophosphate block these stimulations implicating the cholesterol biosynthetic pathway in this up-regulation. However, fluvastatin down-regulates rather than up-regulates COX-2 expression in human umbilical vein endothelial cells [12] and lovastatin reduces PGI₂ production in bovine endothelial cells, human skin fibroblasts and arterial smooth muscle cells [13].

I observed that in rat liver cells, the statins stimulate the release of an essential fatty acid, arachidonic acid (AA) and stimulate production of its metabolite, PGI₂. These stimulations are independent of HMG-CoA reductase activity as measured by the lack of any suppression by mevalonate.

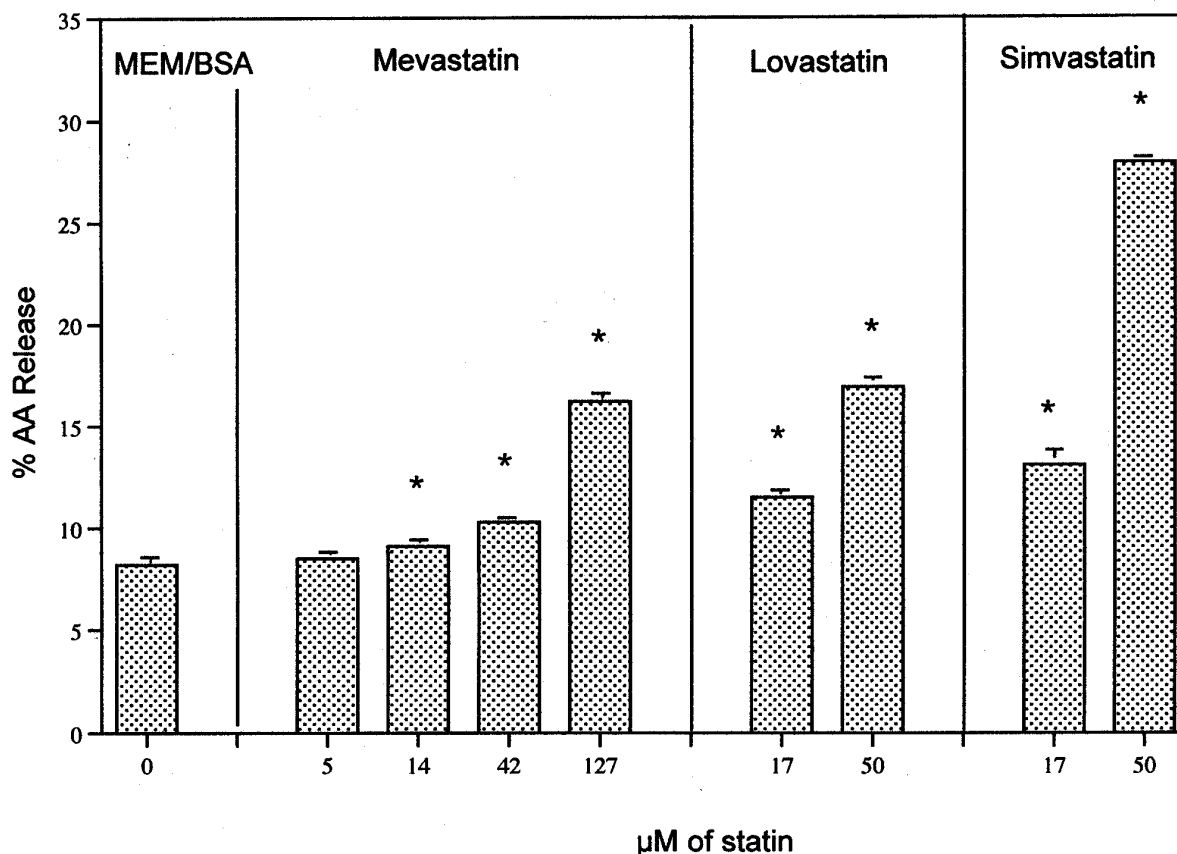


Figure 1

AA release by mevastatin, lovastatin and simvastatin. Cells were incubated with these statins at the concentrations shown for 6 h. The analyses were performed on triplicate culture dishes. * Statistically significant vs MEM/BSA control ($P < 0.05$). Data are representative of several independent experiments.

Results

The release of AA from rat liver cells after 6 h incubation with mevastatin, lovastatin and simvastatin is shown in Fig. 1. An extract of the pharmaceutical product Lipitor® also significantly stimulates AA release (data not shown). The release of AA as a function of time in the presence of 50 μM lovastatin or simvastatin is shown in Fig. 2. Stimulation by lovastatin and simvastatin is observed after as little as 15 min incubation with cells (the time of release with mevastatin was not studied). Preincubation of the cells for 2 h with 1 μM actinomycin did not alter the quantity of AA release stimulated by 6 h incubation with simvastatin [$17.0 \pm 0.30\%$ (5) was released in the absence of actinomycin compared to $17.6 \pm 0.60\%$ (5) released in its presence]. These results indicate that transcription is not

required. I have shown previously that under these conditions, induced PG production is inhibited [14,15]. Nor does mevalonate, the direct product of HMG-CoA reductase, affect the stimulated release by 50 μM simvastatin (Fig. 3).

PGI₂ production is enhanced in cells incubated with lactacystin in the presence of TPA (Fig. 4). Simvastatin, mevastatin and lovastatin enhance the induced PGI₂ levels (Fig. 4). Even at 70 min the quantity of PGI₂ produced by lactacystin plus TPA increased in the presence of 30 μM simvastatin (Fig. 5). The effect of 96 μM mevalonate on simvastatin's amplification of induced PGI₂ in the presence of lactacystin plus TPA is shown in Fig. 6. Mevalonate does not suppress the stimulated PGI₂ production. Meval-

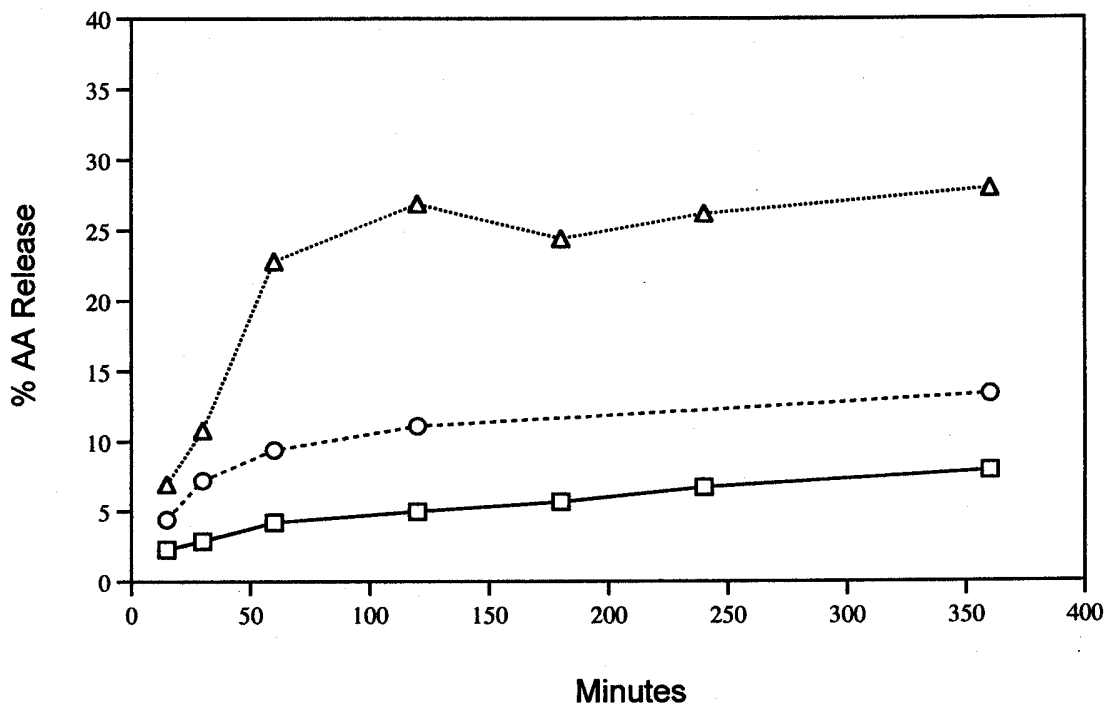


Figure 2

Time course of release of AA after incubation with 50 μ M lovastatin (○), 50 μ M simvastatin (Δ) and the MEM/BSA controls (□). Analyses were performed on triplicate or duplicate dishes. The average value is recorded.

onate amplifies even further the effect of simvastatin on lactacystin plus TPA induced PGI₂ production, suggesting a role for a downstream isoprenoid in the induction pathway (Fig. 6).

Discussion

Mevastatin, lovastatin, simvastatin and atorvastatin stimulate the release of AA from rat liver cells. They do so even after preincubation with actinomycin. Mevalonate, the product of HMG-CoA reductase, does not suppress simvastatin's stimulated AA release. Therefore, it appears that neither transcription nor cholesterol biosynthesis is required for this stimulated AA release. HMG-CoA reductase activity also is not required for up-regulation of induced PGI₂ production by simvastatin.

AA has a number of important bioactivities. It serves as substrate for cyclooxygenases, lipoxygenases and cytochrome P450 epoxygenases. After additional enzyme reactions the products yield a variety of biologically active prostanoids [16]. AA also activates leukocyte NADPH ox-

idase [17], activates PPAR receptors [18], affects H⁺ channel [19], K⁺ channel [20] and Ca²⁺ channel activities [21]. It elevates heat shock gene expression [22], induces apoptosis [23] and inhibits Ca²⁺ induced Ca²⁺ release [24]. In human embryonic kidney cells it down-regulates sterol regulatory element-binding proteins (SREBPs), especially the SREBP-1a isoform [25]. The release of AA after incubation with sulindac and the stimulation of apoptosis *via* conversion of sphingomyelin to ceramide [26] is a mechanism proposed to mediate cancer chemoprevention by nonsteroidal anti-inflammatory drugs [26]. I have postulated that, in addition to nonsteroidal anti-inflammatory drugs, the continuous use of other commonly prescribed drugs that release AA is potentially cancer preventative [27].

The major product of PGH₂ metabolism by these rat liver cells is PGI₂, although 10% or less of the metabolites are PGE₂ and PGF_{2 α} [28]. In this study, only PGI₂ was measured. Although PGI₂ synthase is an induced enzyme [29], the increased PGI₂ production probably reflects COX ac-

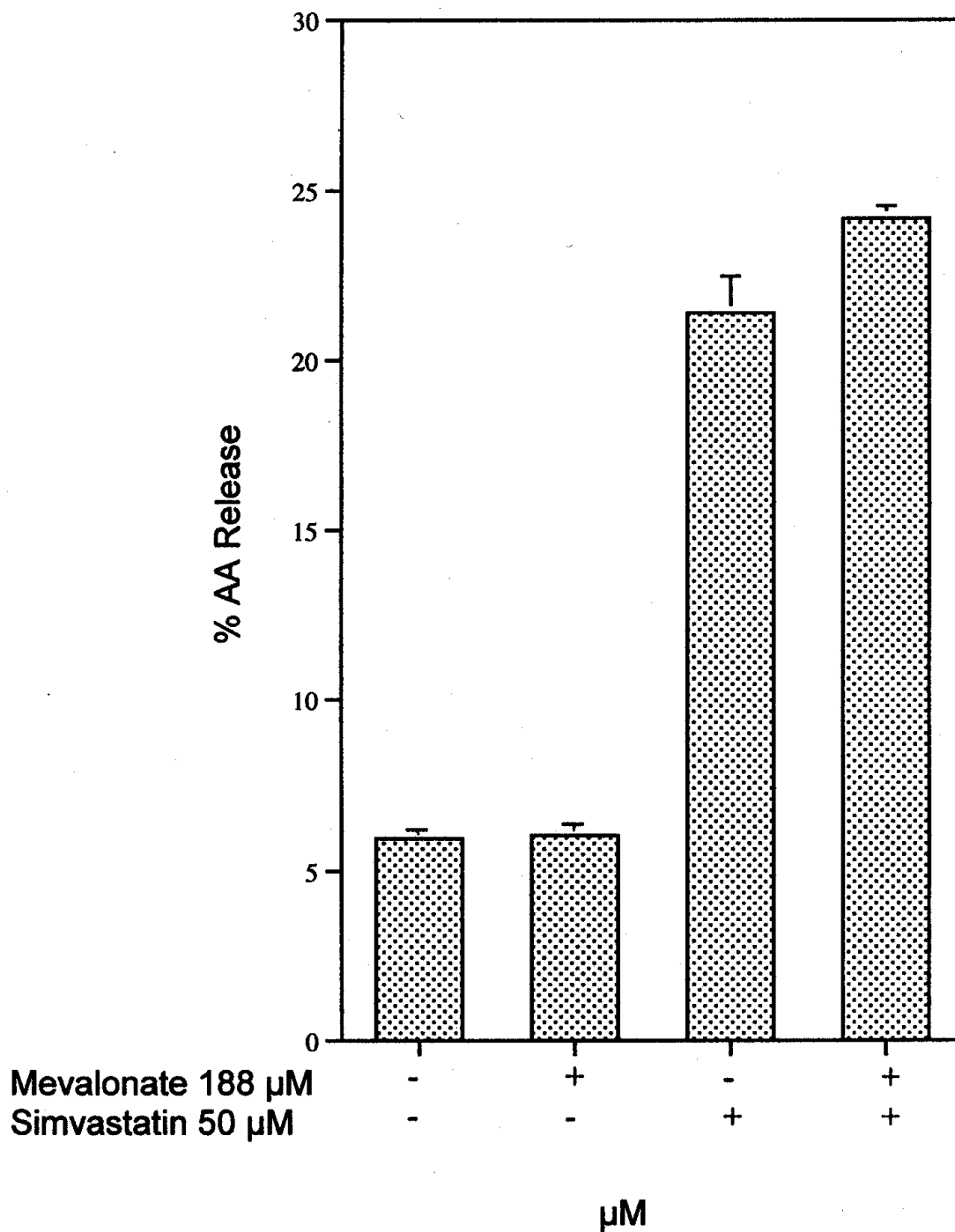


Figure 3
Effect of 188 µM mevalonate on AA release from rat liver cells incubated 6 h in the presence of 50 µM simvastatin. The analyses were performed on triplicate dishes. The bars show the mean values and brackets the SEM. This experiment was repeated two times with similar results.

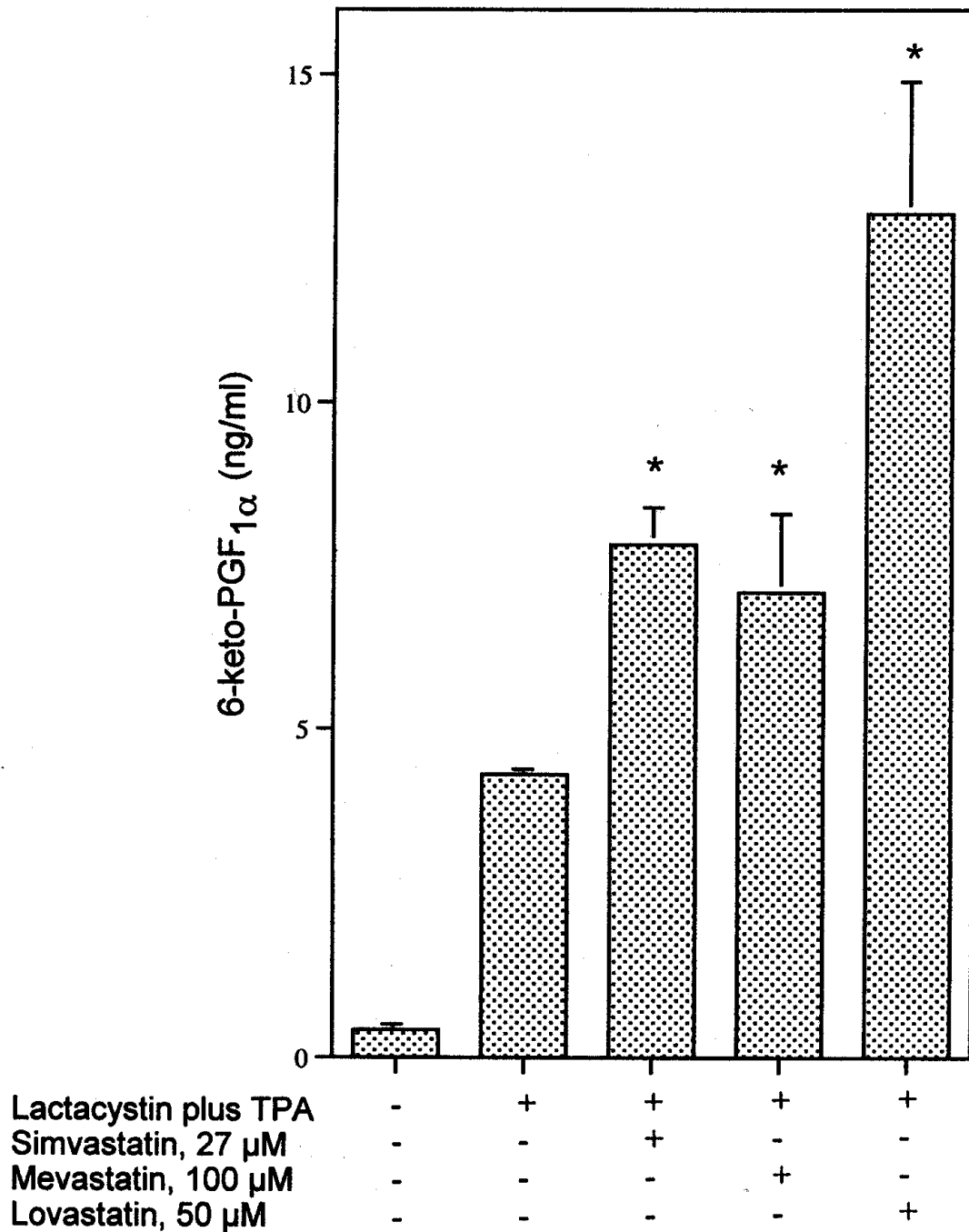


Figure 4

Effect of 27 μM simvastatin, 100 μM mevastatin and 50 μM lovastatin on lactacystin plus TPA induced 6-keto-PGF_{1α} production after 6 h incubation. At the levels tested, simvastatin, mevastatin and lovastatin alone do significantly stimulate basal PGI₂ production, but by < 1 ng/ml. The analyses were performed on triplicate dishes. * Statistically significant vs lactacystin plus TPA (P < 0.05). This experiment was repeated with similar results.

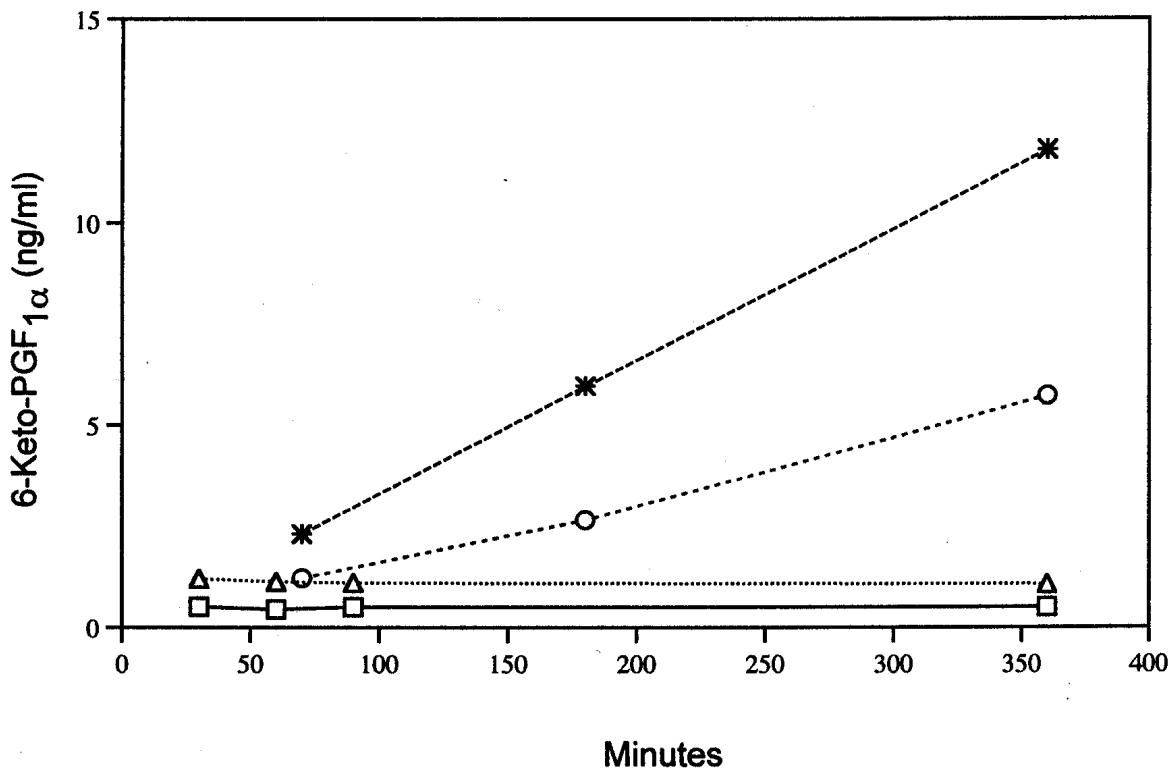


Figure 5

Time course of 6-keto-PGF_{1α} production during incubation with MEM/BSA (□), 30 μM simvastatin (△), lactacystin plus TPA (○) and lactacystin plus TPA plus 30 μM simvastatin (*). Analyses were performed on triplicate or duplicate dishes. The average value is recorded.

tivity [27]. Up-regulation of PGI₂ production is in agreement with the findings of Degreave et al [11] who showed that mevastatin and lovastatin up-regulate COX activity in human aortic smooth muscle cells. However, while up-regulation in the muscle cell system involves cholesterol biosynthesis, the up-regulation observed in rat liver cells does not. Statins also have been shown to down-regulate COX activity in human umbilical vein endothelial cells [12]. In addition to differences inherent in the type of cells being studied by the various investigators, the apparently contradictory findings may also be due to examination of the cells at different stages of growth. The published studies [11–13] use cells at confluent densities and also may have been in the G₀ stage of growth. The rat liver cells studied in this report were at low density (dividing cells).

Since statins activate at μM concentrations, it is likely that the release of AA and effects on PGI₂ production are reflections of interaction with cell membranes.

Conclusions

Statins stimulate AA release from and PGI₂ production in rat liver cells. It is not yet possible to assign a biological consequence to either stimulation. However, in view of the many biological activities of AA, its release could generate the essential fatty acid that is postulated to be a mediator of statin action [10]. Such mediation does not involve cholesterol biosynthesis.

Materials

The C-9 rat liver cell line was purchased from the American Type Culture Collection (Manassa, VA, USA). The

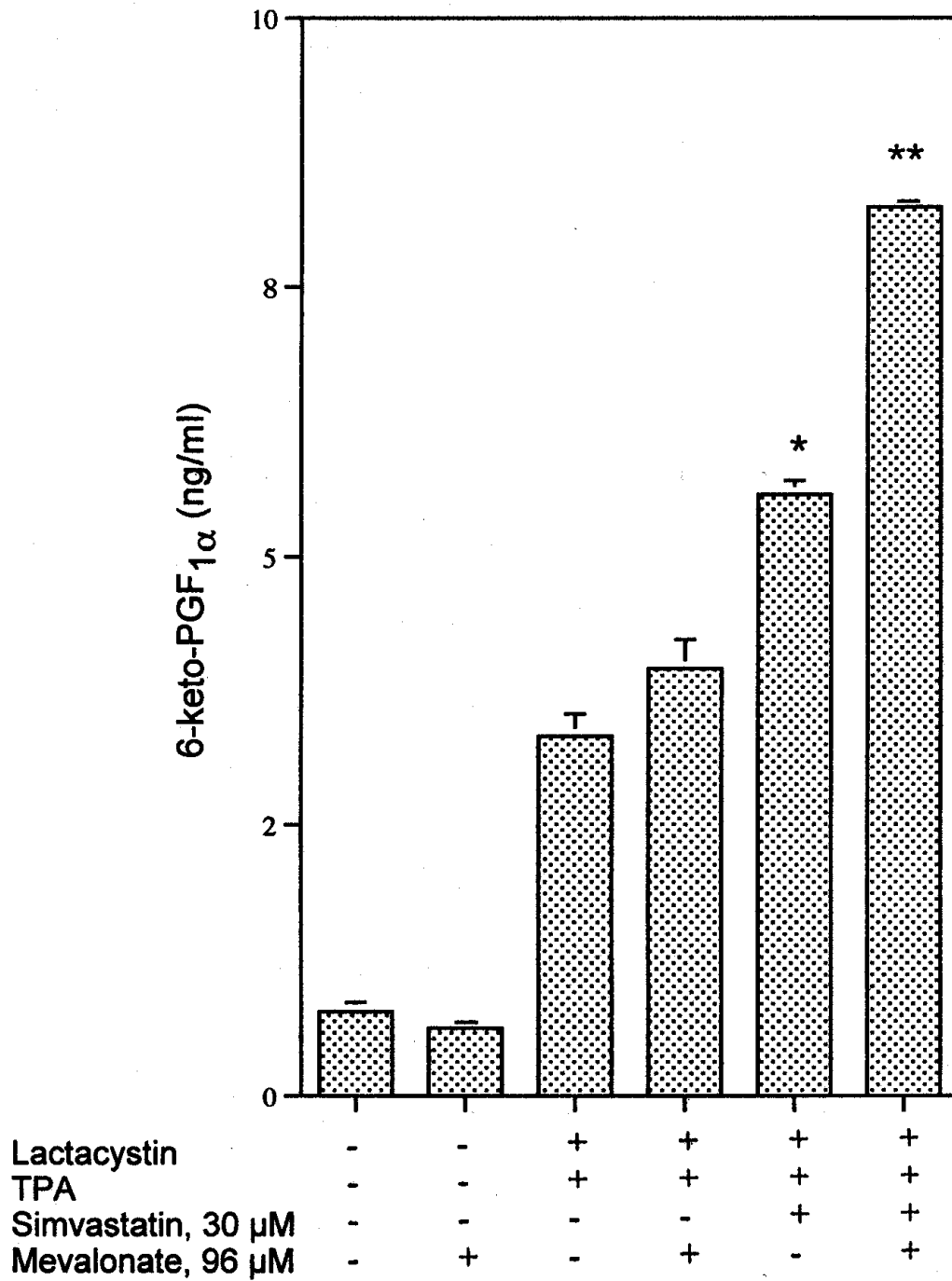


Figure 6
 Effect of 96 μM mevalonate on 6-keto-PGF_{1α} produced by stimulation with 30 μM simvastatin in the presence of lactacystin plus TPA after a 6 h incubation. Analyses were performed on triplicate dishes. * Statistically significant vs lactacystin plus TPA (P < 0.05) ** Statistically significant vs lactacystin plus TPA and simvastatin (P < 0.05).

cells were maintained in Eagle's minimum essential medium (MEM) supplemented with 10% fetal bovine serum. The [³H] AA (91.8 Ci/mmol) was from NEN Life Science Products, Inc., (Boston, MA, USA). Mevastatin, lovastatin, DL-mevalonic acid lactone were from Sigma Chemical Co. (St. Louis, MO, USA). I thank Dr. Gregory R. Mundy, Institute for Drug Development, San Antonio, TX, for the generous supply of simvastatin. For the experiments with atorvastatin, CH₃CH₂OH and CH₃OH extracts of Lipitor[®] tablets were used.

Methods

On the day prior to experiments, the rat liver cells were treated with 0.25% trypsin-EDTA and, after addition of minimal essential media (MEM) containing 10% fetal calf serum, the floating cells were seeded onto 35 mm culture dishes. The plating densities varied from 0.1 to 0.5 × 10⁵ cells/35 mm dish. The freshly seeded cultures were incubated 24 h to allow for cell attachment. After decantation of MEM containing the fetal bovine serum, 1.0 ml fresh MEM containing 10% fetal bovine serum and [³H] AA (0.2 μCi/ml) was added and the cells incubated for another 24 h. The cells were washed 4 times with MEM and incubated for various periods of time with 1.0 ml of different concentrations of each compound in MEM containing 1.0 mg BSA/ml. The culture fluids were then decanted, centrifuged at 2000 × g for 10 min, and 200 μl of the supernate counted for radioactivity. Radioactivity recovered in the washes before the 6 h incubation was compared to input radioactivity to calculate the % radioactivity incorporated into the cells [30].

For PGI₂ production, 1.0 ml of MEM supplemented with 10% fetal bovine serum, void of [³H] AA, was added after the first 24 h incubation. The cells were incubated for another 24 h, washed three times with MEM, then incubated with lactacystin plus TPA and the compounds in MEM containing BSA for various periods of time. The culture fluids were decanted and analyzed for 6-keto-PGF_{1α} the stable hydrolytic product of PGI₂, by radioimmunoassay [31].

Presentation of data

The [³H] AA release is presented as a percentage of the radioactivity incorporated by the cells. Three to six culture dishes were used for each experimental point. The data are expressed as mean values ± SEM (number of dishes). The data were statistically evaluated by unpaired *Student's t*-test. A *P* value < 0.05 was considered significant.

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