Biochemical Pharmacology, Vol. 34, No. 3, pp. 295–300, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

COMMENTARY

PROSTAGLANDINS AND CYCLIC NUCLEOTIDES

MODULATORS OF ARTERIAL CHOLESTEROL METABOLISM

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A plethora of information is available on the role of prostaglandins, specifically prostacyclin (PGI_2), in the control of vascular tone and platelet aggregation in normal and atherosclerotic arteries. For example, it has been posited that PGI_2 synthesis by the vascular endothelium is one of several mechanisms responsible for the thromboresistance of the endothelium of blood vessels [1]. Although the function of PGI_2 in hemostasis has been investigated extensively [2], the potential role of PGI_2 in the modulation of lipid metabolism within vascular cells has not been defined. In recent years, this area of research has received considerable attention particularly since arterial smooth muscle cells derived from atherosclerotic arteries (1) accumulate significant quantities of cholesterol and cholesteryl ester (CE) [3], and (2) produce decreased amounts of PGI_2 during human arteriosclerosis [4, 5].

This mini-review provides a summary of recent findings concerning the role of several eicosanoids in the modulation of CE metabolism in arterial smooth muscle cells. Results of these studies will be used to suggest possible therapeutic applications of PGI_2 and its stable metabolites in the treatment of human arteriosclerosis.

Effects of arterial injury on PGI_2 production and CE metabolism

We know from the work of others that endothelial cells form a thromboresistant surface between the circulating blood and the underlying vessel wall [1, 2]. Among the mechanisms postulated to explain the lack of platelet reactivity to the endothelial surface is the ability of endothelial cells to produce PGI_2 [6]. Recently, Eldor *et al.* [7] reported that, following de-endothelialization of the aorta, PGI_2 production by the vessel wall is low. They observed that neointimal smooth muscle cells soon acquire the capacity to produce PGI_2 following balloon-catheter de-endothelialization, which parallels the initial disappearance of platelets from the damaged vascular surface. Interestingly, additional experiments showed that diet-induced hypercholesterolemia, a risk factor of human arteriosclerosis, inhibits the recovery of PGI_2 by the luminal surface of these injured blood vessels [8]. Eldor *et al.* [8] postulated that cholesterol may increase the thrombogenicity of the arterial wall. Studies by other investigators have suggested that hyperlipemia may increase thrombogenicity as a result of damage to the endothelium with subsequent decreased PGI_2 production [9–11].

While these particular *in vivo* experiments were in progress, some seemingly unrelated studies were conducted which enabled us to develop the following hypothesis, viz. that arterial production of PGI_2 may be involved in the control of arterial lipid metabolism. Based on experiments conducted several years ago, to determine the role of the arterial endothelium in the pathogenesis of arterial CE accumulation, Falcone *et al.* [12] observed that CE was increased significantly in injured aortas of normocholesterolemic and hypercholesterolemic rabbits following arterial injury as compared to corresponding uninjured aortas. Cholesteryl esters preferentially accumulated in re-endothelialized areas and not in the de-endothelialized areas as expected [12]. In additional studies, Hajjar *et al.* [13] observed that, in the injured artery, CE accretion correlated inversely with increased activity of the enzyme responsible for lysosomal CE hydrolysis, i.e. acid cholesteryl ester hydrolase (ACEH). We postulated that these results from injured arteries may be related, in part, to changes in arterial PGI_2 synthesis since (1) neointimal smooth muscle cells of de-endothelialized arteries acquire the capacity to produce PGI_2 in an amount similar to that produced by endothelial cells of the adjacent uninjured artery [7] and (2) de-endothelialized arteries have greater lysosomal CE hydrolytic activity than re-endothelialized arteries [13].

Therefore, the hypothesis was tested that the addition of nanomolar concentrations of PGI_2 may alter CE synthesis and hydrolysis in *intact* arterial smooth muscle cells, and that this eicosanoid may influence cholesterol and CE accumulation in these treated cells [14, 15]. Because it has been well-established by others that several eicosanoids have a known stimulatory effect on the intracellular levels of cyclic AMP in most mammalian cells [16–18], studies were also conducted to investigate a media-

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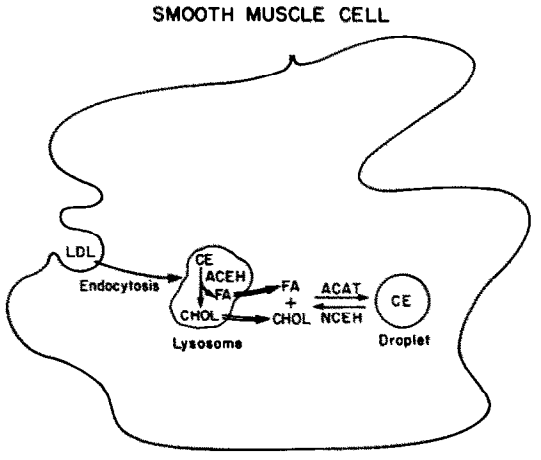

Fig. 1. Cholesteryl ester metabolism in arterial smooth muscle cells. Abbreviations: LDL, low density lipoprotein; CE, cholesteryl esters; CHOL, free cholesterol; ACEH, acid cholesteryl ester hydrolase; ACAT, acyl CoA: cholesterol *O*-acyltransferase; NCEH, neutral cholesteryl ester hydrolase; and FA, fatty acids.

tory role of cyclic nucleotides in the modulation of cholesterol metabolism in those arterial cells which accumulate lipid during vascular disease [14, 15].

The results of these experiments are highlighted in the following section.

Mechanisms of intracellular lipid accumulation

Several major mechanisms may be responsible for the accumulation of cholesterol and CE within arterial intimal smooth muscle cells during atherogenesis. As shown in Fig. 1, increased endocytosis of low density lipoprotein (LDL), which carries free and esterified cholesterol to smooth muscle cells [19], can lead to enhanced intracellular deposition of cholesterol and CE. The CE component of LDL is normally degraded by lysosomes via an (acid) cholesteryl ester hydrolase (EC 3.1.1.13) [20]. However, activity of this enzyme is altered when arterial smooth muscle cells are enriched with lipid, particularly CE. Free cholesterol released from the lysosomal hydrolysis of CE is subsequently re-esterificated in the cytoplasm by microsomal acyl CoA:cholesterol *O*-acyltransferase (ACAT; EC 2.3.1.26) [19]. Cholesteryl ester-rich lipid droplets accumulate in the cytoplasm if the newly formed CE is not hydrolyzed via the cytoplasmic (neutral) cholesteryl ester hydrolase (NCEH; EC 3.1.1.13) [21] or if ACAT activity is enhanced substantially [19].

Activity of sterol-metabolizing enzymes may be affected in cells by prostaglandins [22–24], other hormones [25–28], and lipoproteins [29, 30]. Prostaglandins, in particular, are produced by arterial cells but their production by atherosclerotic vessels is different from normal arteries [4, 31–34], which may influence intracellular cholesterol metabolism.

Difficulties in the interpretation of previous experiments—A need for a new experimental design

To identify a possible mechanism of PGI_2 action on lipid metabolism in arterial smooth muscle cells, a novel approach was needed. Unlike the methods used by others [22–24] who added prostaglandins to arterial homogenates and then assessed enzymatic activity, Hajjar *et al.* [14, 15] incubated the eicosanoids for 2 hr with *intact* cultured smooth muscle cells *prior* to homogenization and assessment of enzymic activities. Significant effects were observed on CE metabolism with *nanomolar* quantities of prostaglandins within a relatively short incubation period using intact cells, but not with homogenized cells. Approaching the problem in this fashion permitted the identification of a mechanism by which concentrations of prostaglandins approaching physiological levels may alter cell metabolism. This would have been difficult to do in previous experiments of others [22–24] where arterial homogenates were prepared first with the subsequent addition of prostaglandins, since membrane-bound phosphorylation cascade sequences can be impaired [14, 35].

Interaction of eicosanoids with CE hydrolytic and synthetic activities

Aside from the studies by Berberian *et al.* [22], Subbiah [23] and Subbiah and Dicke [24] who utilized large quantities of E and F series prostaglandins (0.14 to 1.20 μM) to study their effects on CE hydrolytic and synthetic activities in pigeon or rabbit arterial homogenates, no information was available previously on the effects of prostaglandins, including PGI_2, on CE metabolism under more physiological conditions.

As shown in Fig. 2, 10–20 nM PGI_2 and 55 nM 6-keto PGE_1 significantly enhances lysosomal ACEH activity 4-fold as compared to the untreated controls [14]. Lysosomal ACEH was assayed using labeled cholesteryl oleate given as a phospholipid-digitonin dispersion, a preparation specific only for the assessment of optimal lysosomal CE hydrolysis [21]. Since the effect of these prostaglandins occurred within minutes, it was postulated that enzyme activation may be mediated by stimulation of cyclic nucleotides in the cell. This proposed mechanism seemed reasonable since Claesson [16] and Hopkins and Gorman

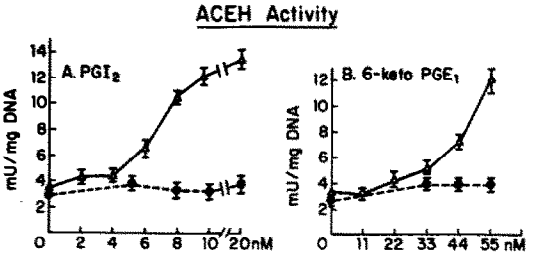

Fig. 2. Effects of (A) prostacyclin (PGI_2) and (B) 6-keto PGE_1 on ACEH activity in smooth muscle cells treated (●-----●) and untreated (△———△) with 0.5 mM dideoxyadenosine (DDA). Each point represents the mean ± S.E.M. for eight separate analyses. A significant increase ($P < 0.05$) in ACEH activity was observed following the addition of 10–20 nM PGI_2 or 55 nM 6-keto PGE_1. [All cells were preincubated with 1 mM methylisobutylxanthine (MIX).] These increases were abolished with pretreatment of the cells with DDA for 20 min prior to the addition of 1 mM MIX and prostaglandins. From Ref. 14.

[17] have demonstrated that PGI_2 can increase cyclic AMP levels in fibroblasts and endothelial cells respectively. Furthermore, others have shown that cyclic AMP will increase CE hydrolytic activities in adipose [36], ovarian [37] and adrenal [38] tissues as well as in macrophages [39]. To test the hypothesis that the prostaglandins alter CE catabolism via intracellular levels of cyclic AMP, 0.5 mM dideoxyadenosine (DDA), an inhibitor of adenylate cyclase activity [40], was preincubated with intact, cultured arterial smooth muscle cells. Following preincubation with DDA, nanomolar quantities of several prostaglandins, including PGI_2, were added to the cultured cells. In cells pretreated with DDA, which maintained cyclic AMP at basal levels, no effect of increasing amounts of various prostaglandins was observed on lysosomal CE hydrolytic activity (ACEH) Fig. 2). Results of Hajjar *et al.* [14] suggested that the prostaglandins stimulate lysosomal CE hydrolytic activity by increasing cyclic AMP in the cell. When 20 μM dibutyryl cyclic AMP was added directly to the cultured smooth muscle cells, a significant 2-fold increase in ACEH activity was observed in the presence of 1.0 mM aspirin which inhibits endogenous production of PGI_2 [14].

Similar to the observations with dibutyryl cyclic AMP, 40 μM sodium arachidonate, the biological precursor to several of the prostaglandins used in these studies, also significantly enhanced CE hydrolytic activity 2-fold [14].

In an experimental approach similar to that taken by Hopkins and Gorman with endothelial cells and Claesson who used fibroblasts [16, 17], Hajjar and Weksler [15] conducted studies to determine if PGI_2 can increase intracellular levels of cyclic AMP in cultured smooth muscle cells (Table 1). As depicted in Table 1, cyclic AMP levels were measured in some cells pretreated with 1 mM methyl-isobutylxanthine (MIX), a potent cyclic AMP phosphodiesterase inhibitor. This agent was added since it can optimally maintain intracellular levels of cyclic AMP. Results of these experiments showed that cyclic AMP could be stimulated and maintained at higher levels by PGI_2 or 6-keto PGE_1 in the presence of MIX during the experimental period as compared to cells not treated with MIX (Table 1). Using renal cells, Rapp *et al.* [41] reported that 6-keto PGE_1 has biological activity. Paradoxically, PGE_1 and PGE_2 did not increase significantly intracellular cyclic AMP levels in arterial smooth muscle cells, albeit endothelial cells have been reported to produce modest amounts of cyclic AMP in response to PGE_1 and PGE_2 [17]. As expected, 6-keto $PGF_{1\alpha}$ did not increase intracellular cyclic AMP levels [15], although a modest effect on ACEH activity was observed with this prostaglandin [14]. The mechanism of action of 6-keto $PGF_{1\alpha}$ on ACEH activity remains undefined. It was concluded that PGI_2 and 6-keto PGE_1 modulate lysosomal CE hydrolysis through intracellular levels of cyclic AMP [14].

Likewise, activities of other lysosomal marker enzymes (acid phosphatase and *N*-acetyl-β-glucosaminidase) were assayed to examine the potential effects of PGI_2 and 6-keto PGE_1 on enzyme activities unrelated to CE metabolism. These lysosomal marker enzyme activities were not altered in arterial smooth muscle cells by PGI_2, 6-keto-PGE_1, PGE_1, or PGE_2 [14], indicating that the effects of PGI_2 and 6-keto PGE_1 on lysosomal (acid) CE hydrolase may be specific.

In an attempt to determine if these eicosanoids affected *cytoplasmic* CE metabolic activity similar to lysosomal CE hydrolysis, Hajjar and Weksler [15] assayed neutral (cytoplasmic) CE hydrolytic activity (NCEH) in cultured arterial smooth muscle cells in the presence of various types of prostaglandins. Results of their studies demonstrated that NCEH activity was significantly enhanced 2-fold by 75 nM PGI_2 (Fig. 3), 40 μM dibutyryl cyclic AMP, and 40 μM arachidonate.

Contrary to the observations that PGI_2 can alter lysosomal and cytoplasmic CE hydrolysis, CE synthesis (ACAT) activity was unaffected by PGI_2 (Fig. 3), dibutyryl cAMP or sodium arachidonate [15]. However, PGE_2 significantly inhibited ACAT activity by 60% (Fig. 3), a finding similar to that reported by Berberian *et al.* [22]. This inhibition occurred in the presence or absence of dideoxyadenosine (DDA). Hajjar and Weksler [15] suggested that the mechanism of action of PGE_2 on ACAT does *not* proceed via intracellular levels of cyclic AMP. Experiments were also conducted to determine if these prostaglandins can alter the activity of the microsomal marker enzyme, viz. neu-

Table 1. Effects of prostaglandins on cyclic AMP levels in arterial smooth muscle cells*

	Cyclic AMP content (pmoles/10^5 cells)		
	No addition	MIX	DDA
PGI_2	15 ± 2†	26 ± 4	2 ± 0.3
6-keto PGE_1	13 ± 1	24 ± 1	2 ± 0.5
6-keto $PGF_{1\alpha}$	3 ± 1	6 ± 1	2 ± 0.4
PGE_1	6 ± 2	6 ± 1	3 ± 0.6
PGE_2	5 ± 1	5 ± 2	3 ± 0.4
Basal levels	4 ± 1	6 ± 1	3 ± 0.3

* The final concentration of prostaglandins in each experiment was 250 nM. The final concentrations of MIX and DDA were 1.0 and 0.5 mM respectively. See Ref. 15 for further details of the experiment.
† Mean ± S.E.; N = 5.

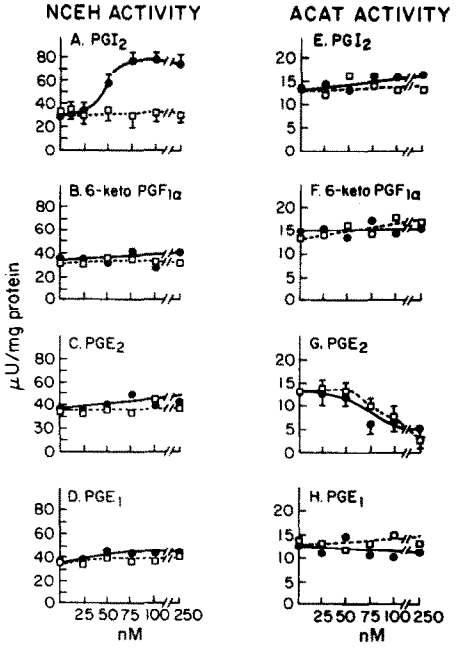

Fig. 3. Effects of prostaglandins on NCEH and ACAT activities in cells treated (□-----□) and untreated (●——●) with 0.5 mM DDA. Each point represents the mean ± S.E.M. from six separate analyses. Cells were incubated with the various concentrations of prostaglandins and 1 mM MIX prior to the assay of enzyme activities. From Ref. 15. [there is a mistake on the ordinate of Panel C (PGE_2) as it appeard originally; 30 should be 20].

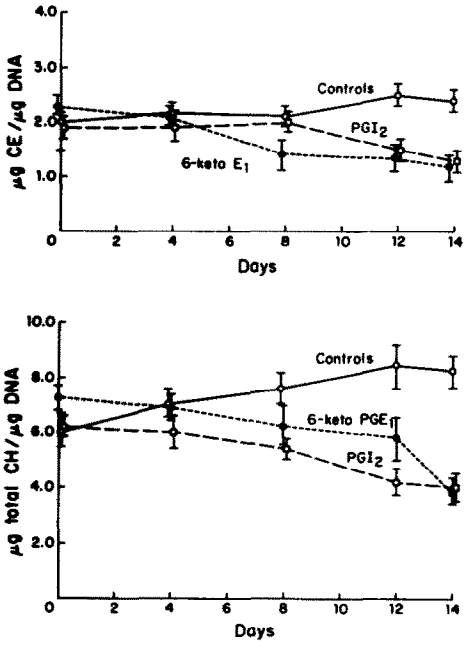

Fig. 4. Effects of PGI_2 and 6-keto PGE_1 on CE and total cholesterol (CH) accumulation. Cells were challenged separately with a 100 nM concentration of the prostaglandins at 0, 2, 6 and 10 days. Five separate analyses were done at 0, 4, 8, 12 and 14 days. Each point represents the mean ± S.E. There was a significant ($P < 0.05$) decrease in total cholesterol and CE accumulation by 14 days in cells treated with PGI_2 or 6-keto PGE_1 as compared to the controls. From Ref. 14.

tral-α-glucosidase. No significant effect could be demonstrated with PGI_2, 6-keto PGE_1, 6-keto $PGF_{1\alpha}$, PGE_1 or PGE_2 on glucosidase activity [15].

Effects of PGI_2 on intracellular levels of cholesterol and CE

Because we [14, 15], Berberian *et al.* [22], Subbiah [23] and Subbiah and Dicke [24] have demonstrated that eicosanoids can alter the activity of arterial CE metabolizing enzymes using various experimental systems, the potential effects of PGI_2 and 6-keto PGE_1 on cholesterol/CE accumulation in arterial smooth muscle cells were examined [14]. These experiments were of particular interest since the effects of prostaglandins on enzyme activity may be short-term and *not* sufficient enough to affect the mass balance of sterols in the cell. As illustrated in Fig. 4, cultured smooth muscle cells from normal rabbit artery were challenged with 100 nM PGI_2 or 100 nM 6-keto PGE_1 four times during a 2-week experimental period. Following a 2-week administration of these prostaglandins, there was a significant reduction in the free and esterified cholesterol content of the prostaglandin-treated cells as compared to the untreated controls. The decrease in cholesterol and CE content appeared to result from excretion of these sterols in the post-culture medium [14]. Since the culture medium contained 25% fetal calf serum [14], these results suggested that high-density lypoproteins (HDL) present in the serum may serve as a "sink" for cholesterol efflux from these arterial cells. Moreover, since it has been shown by others that HDL can stimulate PGI_2 synthesis in arterial cells perhaps by providing arachidonate [42], it may be possible that this lipoprotein may further promote cholesterol efflux by increasing intracellular prostanoid synthesis.

Noteworthy are the recent findings of Tertov and associates [43] who showed that dibutyryl cyclic AMP can reduce significantly the CE content of cultured intimal smooth muscle cells from atherosclerotic lesions by 1.5 to 2-fold. Such observations are consistent with the findings described above which demonstrate that PGI_2 and 6-keto-PGE_1 can reduce intracellular levels of CE. Hajjar *et al.* [14] postulated that the reduction in CE observed in the prostaglandin-treated cells may be due to increased CE hydrolytic activities via enhanced cyclic nucleotide levels.

Correlation between PGI_2, cyclic AMP, and atherosclerosis

Outlined in Fig. 5 is a summary of one pathway by which PGI_2 in particular, may influence the removal of CE from the cell. Briefly, PGI_2 stimulates adenylate cyclase activity, producing increased intracellular levels of cyclic AMP. Cyclic AMP, in turn, may subsequently enhance CE catabolism and cholesterol egress from the cell. Cholesterol egress may then occur due to the presence of intracellular and/or extracellular sterol carrier proteins (SCP) (e.g. HDL) [44].

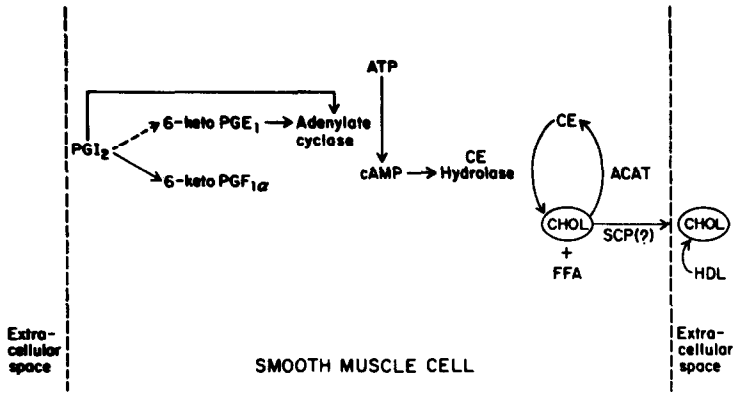

Fig. 5. Hypothetical model describing the action of PGI_2 and 6-keto PGE_1 on the enhancement of cholesterol egress from the cell. From Ref. 44.

Implicit in the hypothesis that PGI_2 can alter intracellular levels of cholesterol and CE in cultured arterial smooth muscle cells is the assumption that similar relationships may occur *in vivo*. To the best of my knowledge, there has not been a definitive study which shows that intravenous administration of PGI_2 can reduce the intracellular concentration of cholesterol and CE in arteries of humans or experimental animals. However, there have been several animal studies which have addressed the issue that PGI_2, hypercholesterolemia, and altered arterial CE metabolism may be related *in vivo*. In rabbits with moderate hypercholesterolemia (serum cholesterol levels of 313 ± 36 mg/dl), a range approximating the levels seen in hypercholesterolemic Western humans, arterial PGI_2 measured at the surface of de-endothelialized arteries was reduced significantly as compared to similar areas in normocholesterolemic rabbits [7, 8]. Likewise, arterial CE hydrolytic activity was also reduced in hypercholesterolemic rabbits as compared to normocholesterolemic rabbits [13]. As expected, hypercholesterolemia leads to increased CE deposition in injured arteries [12]. Thus, significant arterial CE accumulation may result in part from non-activatable CE hydrolase in arterial cells due to altered synthesis of PGI_2 [44].

Finally, experiments highlighted here have been extended recently by others using a different experimental design. In these studies, they have studied the correlation between PGI_2 production and atherogenesis by actually measuring the ability of an *atherosclerotic* artery to produce PGI_2. Cells cultured from atherosclerotic vessels were shown to produce significantly less PGI_2 than cells from normal arteries due to diminished cyclooxygenase and PGI_2 synthetase activities [4, 5]. Lipid peroxides or hydroperoxy acids resulting from lipid accumulation in atherosclerotic vessels may explain the inhibitory effect of PGI_2 synthetic activity [45]. Additional studies using other prostaglandins have supported these tissue culture experiments [46].

Conclusion

We and others have shown that PGE_2 inhibits CE synthetic (ACAT) activity [15, 22], and PGI_2 and 6-keto PGE_1 enhance CE hydrolase activities and cholesterol egress from cultured smooth muscle cells of *normal* arteries [14, 15]. With regard to the several *in vivo* findings discussed previously [7, 8, 12, 13], the *in vitro* results highlighted here suggest that the decreased PGI_2 production and CE hydrolytic activity in the injured arterial wall of hypercholesterolemic rabbits as compared to normocholesterolemic rabbits may explain, in part, the observed changes in cholesterol and CE mobilization and accumulation. Therefore, increased production of PGI_2 and PGE_2 may be inversely correlated with sterol accretion and perhaps atherogenesis *in vivo* [44].

Therapeutic applications of PGI_2

The potential of treating human vascular disease and thrombotic conditions with PGI_2 has been recognized [47]. Beneficial effects of PGI_2 administration have been reported in human advanced arteriosclerosis obliterans [48] and in experimental coronary stenosis [49]. Furthermore, it has been suggested that PGI_2 may help in the management of unstable angina and *early* myocardial infarction by inhibiting platelet accretion at the site of arterial coronary stenosis [50]. Consequently, it would appear from these limited experimental trials that PGI_2 or stable PGI_2 metabolites *may* have a potential role in the treatment of several manifestations associated with atherosclerosis.

Recent experiments by others have been conducted to explore the role of the stable metabolites of PGI_2 catabolism in the control of cholesterol metabolism in cultured arterial smooth muscle cells. For example, Orekhov *et al.* [51] has shown that 6β-PGI_1 and carbocyclin (stable PGI_2 analogues) will lower the CE level in cultured intimal smooth muscle cells from human fatty streaks and atherosclerotic plaques. One can postulate that long-term administration of stable PGI_2 metabolites maybe a more useful means of enhancing cholesterol and CE mobilization out of the arterial "foam" cell during early stages of cardiovascular disease, particularly since pharmacokinetic studies have suggested that PGI_2 itself is too low in the human circulation to mobilize large quantities of accumulating lipid during advanced stages of the disease.

For reasons highlighed here, it is suggested that well-controlled *in vivo* studies are now needed to determine if infusion of stable PGI_2-like analogs will be able to effectively reduce lipid accumulation in the *coronary* arteries. Evidence of this nature will surely offer encouragement for those who have early and moderate stages of arteriosclerosis but who cannot undergo vascular surgery.

REFERENCES

1. S. Moncada, A. G. Herman, E. A. Higgs and J. R. Vane, *Thromb. Res.* **11**, 323 (1977).
2. J. R. Vane and S. Bergstrom, *Prostacyclin*. Raven Press, New York (1979).
3. R. W. St. Clair, in *Atherosclerosis Reviews* (Eds. R. Paoletti and A. M. Gotto, Jr.), p. 61. Raven Press, New York (1976).
4. J. Larrue, C. Leroux, D. Daret and H. Bricaud, *Biochim. biophys. Acta* **710**, 257 (1982).
5. J. Larrue, M. Rigaud, D. Daret, J. Demond, S. Durand and H. Bricaud, *Nature, Lond.* **285**, 480 (1980).
6. B. B. Weksler, A. J. Marcus and E. A. Jaffe, *Proc. natn. Acad. Sci. U.S.A.* **74**, 3922 (1977).
7. A. Eldor, D. J. Falcone, D. P. Hajjar, C. R. Minick and B. B. Weksler, *J. clin. Invest.* **67**, 735 (1981).
8. A. Eldor, D. J. Falcone, D. P. Hajjar, C. R. Minick and B. B. Weksler, *Am. J. Path.* **107**, 186 (1982).
9. M. L. Armstrong, R. E. Peterson, J. C. Hoak, M. B. Megan, F. H. Cheng and W. R. Clarke, *Atherosclerosis* **36**, 89 (1980).
10. T. Henriksen, S. A. Evenson and B. Carlander, *Scand. J. clin. Lab. Invest.* **39**, 361 (1979).
11. A. Nordoy, B. Svensson, D. Wiebe and J. C. Hoak, *Circulation Res.* **43**, 527 (1978).
12. D. J. Falcone, D. P. Hajjar and C. R. Minick, *Am. J. Path.* **99**, 81 (1980).
13. D. P. Hajjar, D. J. Falcone, S. Fowler and C. R. Minick, *Am. J. Path.* **102**, 28 (1981).
14. D. P. Hajjar, B. B. Weksler, D. J. Falcone, J. M. Hefton, K. T. Goldman and C. R. Minick, *J. clin. Invest.* **70**, 479 (1982).
15. D. P. Hajjar and B. B. Weksler, *J. Lipid Res.* **24**, 1176 (1983).
16. H. E. Claesson, *Biochim. biophys. Acta* **518**, 339 (1980).
17. N. K. Hopkins and R. R. Gorman, *J. clin. Invest.* **67**, 540 (1981).
18. L. C. Best, T. J. Martin, R. G. G. Russell and F. E. Preston, *Nature, Lond.* **267**, 850 (1977).
19. J. L. Goldstein and M. S. Brown, *A. Rev. Biochem.* **46**, 897 (1977).
20. N. J. Haley, S. Fowler and C. de Duve, *J. Lipid Res.* **21**, 961 (1980).
21. D. P. Hajjar, C. R. Minick and S. Fowler, *J. biol. Chem.* **258**, 192 (1983).
22. P. A. Berberian, V. A. Ziboh and S. L. Hsia, *Atherosclerosis* **27**, 213 (1977).
23. M. T. R. Subbiah, *Atherosclerosis* **29**, 487 (1978).
24. M. T. R. Subbiah and B. A. Dicke, *Atherosclerosis* **27**, 107 (1977).
25. P. A. Edwards, D. Lemongello and A. M. Fogelman, *J. Lipid Res.* **20**, 2 (1979).
26. M. R. Lakshmanan, C. M. Nepokroeff, G. C. Ness, R. E. Dugan and J. W. Porter, *Biochem. biophys. Res. Commun.* **50**, 704 (1973).
27. C. M. Nepokroeff, M. R. Lakshmanan, G. C. Ness, R. E. Dugan and C. R. Treadwell, *Archs. Biochem. Biophys.* **160**, 387 (1974).
28. G. V. Vahouny, R. Chanderbhan, R. Hinds, V. A. Hodges and C. R. Treadwell, *J. Lipid Res.* **19**, 570 (1978).
29. S. Balasubramanian, J. L. Goldstein, J. R. Faust, G. Y. Brinshede and M. S. Brown, *J. biol. Chem.* **252**, 1771 (1977).
30. S. R. Bates and G. H. Rothblat, *Biochim. biophys. Acta* **360**, 38 (1974).
31. P. A. Berberian, V. A. Ziboh and S. L. Hsia, *J. Lipid Res* **17**, 46 (1976).
32. A. Dembinska-Kiec, T. Gryglewska, A. Zmuda and R. J. Gryglewski, *Prostaglandins* **14**, 1025 (1977).
33. R. J. Gryglewski, A. Dembinska-Kiec, A. Zmuda and T. Gryglewska, *Atherosclerosis* **31**, 385 (1978).
34. H. Sinzinger, P. Clopath, K. Silberbauer and M. Winter, *Experientia* **36**, 321 (1980).
35. L. J. Ignarro, in *Lysosomes in Biology and Pathology* (Eds. J. T. Dingle and R. T. Dean), Vol. 4, p. 481. North Holland Elsevier, New York (1975).
36. J. C. Khoo, D. Steinberg, J. J. Huang and P. R. Vagelos, *J. biol. Chem.* **251**, 2882 (1976).
37. F. A. Kuehl, *Prostaglandins* **5**, 325 (1974).
38. A. Naghskinek, C. R. Treadwell, L. Gallos and G. V. Vahouny, *Biochem. biophys. Res. Commun.* **61**, 1076 (1974).
39. J. C. Khoo, E. M. Mahoney and D. Steinberg, *J. biol. Chem.* **256**, 12659 (1981).
40. R. J. Haslam, A. M. L. Davidson and J. V. Desjardins, *Biochem. J.* **176**, 83 (1978).
41. N. S. Rapp, T. V. Zenser and B. B. Davis, *Biochim. biophys. Acta* **673**, 163 (1981).
42. L. N. Fleisher, A. R. Tall, L. D. Witte, R. W. Miller and P. J. Cannon, *J. biol. Chem.* **257**, 6653 (1982).
43. V. V. Tertov, A. N. Orekhov, V. S. Repin and V. N. Smirnov, *Biochem. biophys. Res. Commun.* **109**, 1228 (1982).
44. D. P. Hajjar, *Advances in Cyclic Nucleotides and Protein Phosphorylation Research* (Eds. P. Greengard, G. A. Robison, R. Paoletti and S. Nicosia). Vol. 17, p. 605, Raven Press, New York (1984).
45. S. Moncada, R. J. Gryglewski, S. Bunting and R. J. Vane, *Prostaglandins* **12**, 715 (1976).
46. S. A. Singh, F. A. Shamgar and A. J. Day, *Expl. molec. Path.* **37**, 74 (1982).
47. B. B. Weksler, in *Progress in Hemostasis and Thrombosis* (Ed. T. H. Spaet), Vol. 6, p. 113. Grune & Stratton, New York (1982).
48. A. Szczeklik, S. Skawinski, P. Gluszko, R. Nizankowski, J. Szczeklik and R. J. Gryglewski, *Lancet* **1**, 111 (1979).
49. J. W. Aiken, R. R. Gorman and R. J. Shebuski, *Prostaglandins* **17**, 483 (1979).
50. G. Bergman, L. Atkinson, P. J. Richardson, K. Daly, M. Rothman, G. Jackson and D. E. Jewitt, *Lancet* **1**, 569 (1981).
51. A. N. Orekhov, V. V. Tertov and V. N. Smirnov, *Lancet* **1**, 521 (1983).