ELCOSANOIDS, APOLIPOPROTEINS, LIPOPROTEIN PARTICLES, AND ATHEROSCLEROSIS

EICOSANOIDS, APOLIPOPROTEINS, LIPOPROTEIN PARTICLES, AND ATHEROSCLEROSIS

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PREFACE

Plasma lipoproteins constitute a unique macromolecular system of lipid-protein complexes responsible for the transport of lipids from their sites of origin to their sites of utilization either as metabolic fuel or as structural components of cell membranes. Although studies on the role of lipoproteins in the mechanism of lipid transport are meritorious in their own right, the ever-increasing interest in chemical and functional properties of this remarkable class of conjugated proteins stems from the impressive evidence of their direct involvement in the genesis and development of atherosclerotic lesions. The initial emphasis on neutral lipids and phospholipids as the most characteristic constituents of operationally-defined lipoprotein classes has shifted in recent years to their protein moieties or apolipoproteins. The discovery of a number of apolipoproteins and characterization of familial hypolipoproteinemias as apolipoprotein deficiency disorders indicated that apolipoproteins play an essential role in maintaining the structural stability and integrity of lipoprotein particles. In addition to their role in the formation of lipoproteins, apolipoproteins were shown to perform a variety of functions in metabolic conversion of lipoproteins and their interactions with cellular surfaces. Results from several laboratories have demonstrated that the chemical and metabolic heterogeneity of operationally-defined lipoprotein classes is due to the presence of several discrete lipoprotein particles with similar physical properties but different and characteristic apolipoprotein composition. Thus, the apolipoproteins have emerged not only as essential structural and functional constituents of lipoproteins but also as unique chemical markers for identifying and classifying lipoprotein particles. The crowning achievement of this "apolipoprotein phase" of lipoprotein research has been the elucidation of the primary structure of almost all recognized apolipoproteins including apolipoprotein B.

These developments, providing new information on the role of apolipoproteins in normal and impaired lipid transport processes and atherogenesis were presented and discussed expertly by the participants of the Fourth International Colloquium on Eicosanoids, Apolipoproteins, Lipoprotein Particles and Atherosclerosis held at the University of Brussels, Belgium, under the sponsorship of the "Fondation de Recherche sur l'Athérosclérose".

The opening topic of this volume was devoted to recent advances in studies on the interactions between lipoproteins and eicosanoids and their significance in atherogenic processes. The next major topics included genetic disorders of lipid transport and their effect on coronary artery disease, new findings on primary, secondary and tertiary structures of apolipoproteins and lipoproteins, and the structure, genetics and potential function of serum amyloid A protein in lipid transport. Another timely

topic covered the structure and role of lipid transfer proteins in modulating lipid composition and metabolic fate of lipoprotein particles. The topic on apolipoprotein defined lipoprotein particles presented preliminary evidence for the chemical and functional specificities of discrete ApoA- and ApoB-containing lipoproteins. The closing topic was devoted to the chemical and metabolic abnormalities of lipoproteins in hypertriglyceridemic states.

We hope this volume will be of interest to all investigators in the area of lipoprotein and atherosclerosis research as a useful review of recent accomplishments and a stimulating guide for future studies.

P. Alaupovic

C.L. Malmendier

CONTENTS

EICOSANOIDS

GENETICS OF APOLIPOPROTEINS

STRUCTURE OF APOLIPOPROTEINS AND LIPOPROTEINS

ACUTE PHASE APOLIPOPROTEINS

LIPID TRANSFER PROTEINS

LIPOPROTEIN PARTICLES

HYPERTRIGLYCERIDEMIA

PROSTACYCLIN, EDRF AND ATHEROSCLEROSIS

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Introduction

The main metabolic product of the fatty acid arachidonic acid (AA) in vascular tissue is prostacyclin, a potent vasodilator and inhibitor of platelet aggregation (1). In contrast, in platelets AA is mainly converted to thromboxane A_2, which is a potent vasoconstrictor and inducer of platelet aggregation. An imbalance in the formation or the interaction between these two AA metabolites has been implicated in the underlying process of various thrombotic conditions as well as of atherosclerosis (2).

Another vasodilator mechanism, independent of prostacyclin and mediated by vascular endothelial cells, was described in 1980. This vasodilatation has been shown to be mediated by the release of a humoral agent which became known as endothelium-derived relaxing factor (EDRF) (3). We have identified the chemical nature of EDRF as nitric oxide (NO) (4). Prostacyclin and NO both inhibit platelet aggregation, moreover NO is a potent inhibitor of platelet adhesion. It is likely that, in addition to their role as regulators of vascular smooth muscle tone, prostacyclin and NO also synergise to regulate platelet-vessel wall interactions.

Vascular production of prostacyclin

Prostacyclin is generated by blood vessel microsomes or fresh vascular tissue from AA itself or from the unstable intermediate in AA metabolism, the prostaglandin (PG) endoperoxide, PGH_2. In common with other prostaglandins, prostacyclin release can be stimulated by mechanical, immunological or chemical means, as well as by trauma. Prostacyclin is a vasodilator and the most potent naturally-occurring inhibitor of platelet aggregation yet discovered. It is chemically unstable, with a half-life of 2-3 min, breaking down to 6-keto-$PGF_{1\alpha}$ (for review see 2).

The inhibition of platelet aggregation by prostacyclin is correlated with an activation of the adenylate cyclase system, leading to a substantial rise in platelet intracellular cyclic AMP levels. Other properties of prostacyclin include cytoprotection, enhanced fibrinolysis and stimulation of cholesterol metabolism (for review see 5). Unlike most properties of prostacyclin, cytoprotection is independent of an effect on cyclic AMP.

The ability of medium sized vessels to synthesise prostacyclin is greatest at the intimal surface and decreases progressively towards the adventitia (6). Production of prostacyclin by vascular cells in culture shows that endothelial cells are the most active producers of prostacyclin, although smooth muscle cells also convert AA to prostacyclin (7). Stripping of the endothelium from rabbit aorta in vivo removes virtually all ability of the luminal surface to produce prostacyclin from exogenously-added AA (8). Recent studies have shown that although the subendothelium has a significant capacity to produce prostacyclin, this capacity is only expressed briefly (9).

Prostacyclin and atherosclerosis

There is evidence to suggest that a number of diseases, such as atherosclerosis, are associated with an imbalance in the prostacyclin/TXA_2 system. A decrease in prostacyclin formation by atherosclerotic vascular tissue has been demonstrated both in experimental animals and in man. It has recently been shown that aortae from rabbits made atherosclerotic by cholesterol feeding show a transient increase in prostacyclin production followed by a long-lasting reduction (10). Moreover, smooth muscle cells obtained from atherosclerotic lesions and cultured in vitro consistently produce less prostacyclin than normal vascular smooth muscle cells. These findings probably reflect the increased generation by atherosclerotic vessels of lipoxygenase products, which are selective inhibitors of prostacyclin formation (11).

Atherosclerosis is not invariably associated with reduced generation of prostacyclin as there have been reports of increased release of prostacyclin, or a metabolite, both in experimental models of atherosclerosis (12) and in patients with severe diffuse atherosclerosis (13). This may be the result of the release from activated platelets of factors, such as 5-hydroxytryptamine and platelet-activating factor, with potential prostacyclin-releasing properties.

Prostacyclin has recently been shown to have a regulatory role in aortic cholesterol metabolism. As an atherosclerotic lesion progresses, cholesterol and cholesteryl esters are deposited in the extracellular matrix of the aortic smooth muscle cells as well as in lysosomal and cytosolic compartments of these cells. In cultured smooth muscle cells from the thoracic aorta of rabbits, prostacyclin at low concentrations increases the activity of the enzymes that metabolise cholesteryl esters, while PGE_2 inhibits cholesteryl ester synthetic activity (14). In human atherosclerotic cells in culture, cholesteryl ester metabolism is enhanced by two stable prostacyclin analogues so that the triglyceride and cholesteryl ester levels in the cells are substantially reduced (15).

Prostacyclin can also inhibit mobilisation of fibrinogen-binding sites on human platelets in vitro, which thus may limit the extent of fibrinogen-platelet interactions. In addition, prostacyclin enhances fibrinolytic activity in the canine lung, in human subjects and in human skin fibroblasts where it induces the protease plasminogen activator, a process which could contribute to its long-term clinical actions in chronic obstructive diseases of the circulation (for references see 16).

A new, potentially anti-atherosclerotic effect of prostacyclin has been described by Willis and co-workers (17) who showed that prostacyclin inhibits the release of mitogenic activity ('growth factors') from stimulated human platelets. Such growth factors are thought to mediate the progression of atherosclerosis by promoting smooth muscle cell and fibroblast proliferation (18).

Cigarette smoking is a well-recognised risk factor in atherosclerosis and is known to increase platelet reactivity and to reduce both basal and stimulated levels of urinary 6-keto-$PGF_{1\alpha}$ in man. Prostacyclin formation by kidney microsomes and rabbit isolated hearts is reduced by nicotine. Umbilical arteries from mothers who smoke produce significantly less prostacyclin than those from non-smoking control subjects (for references see 16).

Prostaglandin production by vascular tissue and platelets is also altered in a number of diseases associated with increased risk of arterial thrombosis, for example diabetes and thrombotic thrombocytopenic purpura. In general it seems that in diseases where there is a tendency for thrombosis to develop, TXA_2 production is increased, or prostacyclin production reduced, or both, whilst the opposite is found in some diseases associated with an increased bleeding tendency (for review see 19).

The use of prostacyclin, as a stable freeze-dried preparation (Epoprostenol), has been studied in several clinical conditions (for reviews see 19,20). In addition to its use in extracorporeal circulation systems such as cardiopulmonary bypass operations, renal dialysis and charcoal haemoperfusion, prostacyclin has been shown to be effective in the treatment of atherosclerotic peripheral vascular disease and Raynaud's syndrome. It is also being tested for the treatment of other thrombotic conditions such as stroke and myocardial infarction. Prostacyclin has been used in patients with pulmonary hypertension and has been found to have pulmonary haemodynamic effects similar to those of hydralazine and nifedipine. What is becoming clear is that the efficacy of prostacyclin, particularly its long-lasting effect in certain clinical conditions like peripheral vascular disease and Raynaud's syndrome, cannot be explained solely in terms of its short-lasting vasodilator and anti-aggregatory actions. Consequently, interest is being focused on the other activities of prostacyclin such as cytoprotection, fibrinolysis and stimulation of cholesterol metabolism.

Biological properties of endothelium-derived relaxing factor

The role of the endothelium in the relaxation of vascular tissues was discovered accidentally when it was observed that strips or rings of contracted rabbit aorta (RbA) exhibited great variation in their ability to relax to acetylcholine (ACh). Although a potent vasodilator *in vivo*, ACh is frequently inactive or occasionally produces a small contraction of vascular strips or rings *in vitro*. The explanation of this phenomenon was provided by Furchgott and Zawadzki (3) when they showed that ACh-induced vascular relaxation was dependent on the presence of the intact endothelium. Endothelial cells stimulated with ACh release a factor (later called endothelium-derived relaxing factor, EDRF) which diffuses to the underlying smooth muscle to cause relaxation.

It is now known that, in addition to ACh, many other vasodilators are also endothelium-dependent. These include adenine nucleotides, thrombin, substance P, the calcium ionophore A23187 (calcimycin), vasopressin, vasoactive intestinal polypeptide (VIP), pancreozymin, calcitonin gene-related peptide, bradykinin, bee venom (melittin) and saturated and unsaturated fatty acids (21). Other substances, however, including the nitrovasodilators, atrial natriuretic factor, bovine retractor-penis inhibitory factor and prostacyclin, cause vascular relaxation by endothelium-independent mechanisms.

The humoral nature of EDRF was first demonstrated with a variety of

donor/detector systems (for reviews see references 21 and 22). Such bioassay methods revealed that EDRF was a labile substance with a half-life of between 3 and 50 seconds. It also became apparent that a variety of seemingly unrelated compounds could inhibit endothelium-dependent relaxation in intact vascular preparations.

Later it was shown that superoxide anions (O_2^-) contribute to the instability of EDRF (23,24). Superoxide dismutase (SOD), by inactivating O_2^-, potentiates the activity of EDRF. It was also demonstrated that compounds such as phenidone, BW755C, dithiothreitol, hydroquinone and other redox compounds inhibit the action of EDRF via the generation of O_2^- (25). Haemoglobin, however, is another inhibitor of the action of EDRF and of endothelium-dependent relaxation which acts by binding to the EDRF molecule rather than via a mechanism involving the generation of O_2^- (26).

EDRF is also an inhibitor of platelet aggregation. We have observed EDRF-induced inhibition of platelet aggregation in human platelet-rich plasma and washed platelets, induced by collagen, U46619, ADP and thrombin, and have shown that EDRF is equi-active against all these aggregating agents (27,28). EDRF also induces disaggregation of platelets aggregated with collagen and with U46619.

The inhibitory action of EDRF on platelet aggregation can be clearly differentiated from that of prostacyclin. Unlike prostacyclin, the effect of EDRF on platelets is potentiated by SOD and M&B 22948, a selective inhibitor of the cyclic GMP phosphodiesterase, and inhibited by Fe^{2+}, haemoglobin and hydroquinone. These effects are consistent with those observed on vascular strips. Moreover, the inhibitory effect on platelet aggregation is accompanied by an increase in platelet cyclic GMP. The duration of the effect of EDRF on platelets is short, with a half-life of 2 minutes, while that of prostacyclin has a half-life of 4 minutes.

A number of authors have demonstrated a rise in smooth muscle cell cyclic GMP levels associated with endothelium-dependent relaxation or with EDRF-induced vascular relaxation. EDRF, in common with the nitrovasodilators, has been shown to activate soluble guanylate cyclase from smooth muscle cells (29). Endothelium-dependent relaxation and the action of EDRF on platelets are prevented by agents that interfere with the activation of this enzyme, such as haemoglobin and methylene blue (30). These data support the concept that stimulation of soluble guanylate cyclase underlies the vascular relaxant and anti-aggregatory actions of EDRF.

Chemical identification of EDRF

Widespread speculation about the chemical nature of EDRF developed soon after its discovery. In 1986, however, Furchgott (31) and Ignarro et al. (32) independently suggested that EDRF may be NO or a closely-related species. We decided to investigate whether EDRF was indeed NO by comparing first, the pharmacological profile of EDRF and authentic NO on vascular strips and on platelets, and second by measuring directly the release of NO from porcine aortic endothelial cells in culture (4).

Both EDRF and NO caused a relaxation of the vascular strips which declined at the same rate during passage down the cascade. Furthermore, the rate of decay was slower, but similar for both compounds, during transit in polypropylene tubes. Both compounds also inhibited platelet aggregation (27), induced the disaggregation of aggregated platelets (28) and inhibited platelet adhesion (33). Moreover,

their biological half-life as inhibitors of platelet aggregation was similar (27).

The actions of EDRF and NO on vascular strips and on platelets were similarly potentiated by SOD and cytochrome c and inhibited by Fe^{2+} and some redox compounds (27,34). Furthermore, the potency of redox compounds as inhibitors of EDRF- and NO-induced vascular relaxation was attenuated by SOD to a similar extent. Haemoglobin also inhibited the effect of EDRF and NO through a mechanism not involving O_2^-. Finally, direct measurements of cyclic GMP, or studies with selective inhibitors of its specific phosphodiesterase, have demonstrated that both compounds act on vascular smooth muscle and platelets via the stimulation of soluble guanylate cyclase and elevation of cyclic GMP.

Nitric oxide may be measured directly as the chemiluminescent product of its reaction with ozone. Using this method we have shown that the concentrations of bradykinin which induce the release of EDRF from endothelial cells in culture also cause a concentration-dependent release of NO (4). Moreover, we have established that the amounts of NO released by the cells are sufficient to account both for relaxations of the vascular strips and for the anti-aggregating and anti-adhesive activity of EDRF (4,28,33). Recently, we have also observed that the vascular relaxing activity released from fresh, perfused arteries of the rabbit, cat and dog by a number of agents, including ACh, substance P and bradykinin, is accounted for by the amounts of NO released (unpublished observations).

All this pharmacological and biochemical evidence clearly demonstrates that EDRF is NO and that it fulfils all the criteria necessary to be classified as a biological mediator (35).

Interactions between NO and prostacyclin

Prostacyclin and NO potentiate each other as inhibitors of platelet aggregation and inducers of platelet disaggregation (28). The supernatants of endothelial cells stimulated with low concentrations of bradykinin contain amounts of NO and prostacyclin too low to explain the anti-aggregating activity observed when these supernatants are added to platelets. This activity is, therefore, the result of a synergistic interaction between NO and prostacyclin. As a result, we have suggested that the very low concentrations of prostacyclin found in plasma may have a physiological effect in regulating platelet aggregability if acting on a background of NO release (36).

Interestingly, NO differs from prostacyclin in that it is also an effective inhibitor of platelet adhesion. The fact that we did not observe a synergistic interaction between these two compounds on platelet adhesion (33) suggests that the physiological process of platelet adhesion and repair of the vessel wall may proceed under circumstances in which both substances, acting in concert, are exerting a powerful anti-thrombotic action.

Prostacyclin and NO are both powerful vasodilators. The interaction between NO and prostacyclin as vasodilators remains to be studied. Preliminary evidence from our laboratory has not shown synergy between NO and prostacyclin in the rabbit mesenteric artery strip (unpublished observations).

The subcellular mechanisms underlying the actions of NO and prostacyclin are the cyclic GMP and the cyclic AMP systems, respectively. It is interesting that in some situations, such as inhibition of platelet

aggregation and induction of disaggregation, there is a synergy between the two while in others, such as inhibition of platelet adhesion, there may even be some antagonism (33).

Physiological and pathological implications of EDRF

Although the release of EDRF or NO has not been demonstrated in vivo, endothelium-dependent vasodilatation has been demonstrated in vivo in a number of species. The changes in diameter which follow changes in blood flow in a number of perfused artery preparations (37-39) are endothelium-dependent and it has been suggested that endothelium-dependent relaxation coordinates increased flow responses through vascular beds (40).

Endothelium-dependent relaxation, or the release of NO, may be impaired in atherosclerosis, as a decrease in the ability of the vascular endothelium to release EDRF (41) or a decrease in the endothelium-dependent relaxation (42,43) has been demonstrated in vascular tissue obtained from rabbits with dietary-induced atherosclerosis. Furthermore, low density lipoproteins, which are associated with the development of atherosclerosis, have been shown to inhibit endothelium-dependent relaxation (44).

Studies in strips of human coronary artery with severe atherosclerosis have shown reduced endothelium-dependent relaxation in response to substance P, bradykinin and A23187 (45). In patients with moderate to severe atherosclerosis, infusions of ACh caused dose-dependent coronary vasoconstriction whereas dose-dependent vasodilatation was observed in control subjects (46). In contrast, glyceryl trinitrate caused dilatation in both normal and atherosclerotic subjects.

Reduced endothelium-dependent relaxation in animals with spontaneous or experimentally-induced hypertension has been reported by some workers (47-51). Restoration of blood pressure to normal can reverse this impairment in the endothelium-dependent response (52,53).

Reduced generation of NO by endothelial cells could play a role in the genesis of coronary vasospasm since endothelial damage renders vessels susceptible to local spasm in experimental animals (54). Inhibition of NO by haemoglobin could also play a role in the vasospasm that follows subarachnoid haemorrhage, which has long been suspected to be mediated by some product of lysed red blood cells (55).

If there is indeed a decrease in the release of NO in the vascular endothelium during hypertension or atherosclerosis, it will be very important to investigate the mechanism by which this takes place. Lipid peroxides and O_2^-, which inhibit the synthesis of prostacyclin (11) and play a role in the destruction of NO (23,24), have long been implicated in the genesis of different types of cardiovascular disease, including atherosclerosis. It would not be surprising if the biochemical basis of their action is related to these effects. Interestingly, O_2^- ions are responsible for the conversion of the ACh-induced cerebral arteriolar dilation to vasoconstriction during an infusion of norepinephrine (56).

Conclusions

Nitric oxide is an important mediator in the vessel wall and may be considered to be the endogenous nitrovasodilator.