Platelet Dysfunction in Type 2 Diabetes

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Insulin resistance is a uniform finding in type 2 diabetes, as are abnormalities in the microvascular and macrovascular circulations. These complications are associated with dysfunction of platelets and the neurovascular unit. Platelets are essential for hemostasis, and knowledge of their function is basic to understanding the pathophysiology of vascular disease in diabetes. Intact healthy vascular endothelium is central to the normal functioning of smooth muscle contractility as well as its normal interaction with platelets. What is not clear is the role of hyperglycemia in the functional and organic microvascular deficiencies and platelet hyperactivity in individuals with diabetes. The entire coagulation cascade is dysfunctional in diabetes. Increased levels of fibrinogen and plasminogen activator inhibitor 1 favor both thrombosis and defective dissolution of clots once formed. Platelets in type 2 diabetic individuals adhere to vascular endothelium and aggregate more readily than those in healthy people. Loss of sensitivity to the normal restraints exercised by prostacyclin (PGI_2) and nitric oxide (NO) generated by the vascular endothelium presents as the major defect in platelet function. Insulin is a natural antagonist of platelet hyperactivity. It sensitizes the platelet to PGI_2 and enhances endothelial generation of PGI_2 and NO. Thus, the defects in insulin action in diabetes create a milieu of disordered platelet activity conducive to macrovascular and microvascular events.

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Insulin resistance (IR) (i.e., resistance to insulin-stimulated glucose uptake) presents in a majority of individuals with type 2 diabetes; it appears to be a common precursor of both diabetes and macrovascular disease (1). IR is a multisystem disorder that is associated with multiple metabolic and cellular alterations. Factors that contribute to IR are genetics, obesity, physical inactivity, and advancing age (2). Metabolic disturbances that commonly occur in patients with IR are atherogenic dyslipidemia, hypertension, glucose intolerance, and a prothrombotic state (1,2).

Atherogenic dyslipidemia is characterized by three lipoprotein abnormalities: elevated VLDL, small LDL particles, and decreased HDL cholesterol levels (the lipid triad), also named the atherogenic lipoprotein phenotype (2). This triad is the hallmark of people with diabetes and IR and appears to be an atherogenic phenotype independent of elevated levels of LDL cholesterol (2). As a corollary, most patients with IR have this phenotype even if they are not diabetic, and it may precede the development of diabetes by many years (2).

Hypertension, a well-established risk factor for macrovascular events, is also associated with IR. In fact, a direct relationship between plasma insulin concentration and blood pressure has been noted (1). Although the list of multifactorial events that link hypertension and IR is growing (3), currently, the emphasis rests on the role of the endothelial cell.

Hypertension is a component of the metabolic syndrome, which occurs in many patients with the lipid triad (2,3). IR appears to underlie the metabolic syndrome, which consists of the coexistence of the lipid triad, elevated blood pressure, IR, and a prothrombotic state in a single person (2).

This prothrombotic state is a more recently recognized component of the metabolic syndrome; people with the metabolic syndrome exhibit a pattern of coagulation factors that promote thrombosis or retard thrombolysis (2,4). The prothrombotic state is characterized by increased fibrinogen levels (5), increased plasminogen activator inhibitor (PAI)-1 (6), and different abnormalities in platelet function (4,7).

Thus, evidence that diabetes belongs to a special category of risk factors for vascular disease continues to grow. This risk is partly due to the pernicious effects of persistent hyperglycemia, but clearly other factors involved beyond glycemia contribute to the IR syndrome (8). Defining IR simply on the basis of failure of insulin to regulate glucose utilization falls short of the myriad effects caused by defective insulin action. A better definition of insulin resistance would be "the metabolic state in which the measured tissue response to insulin is less than that expected for the apparently available insulin." This response applies to metabolic fuels and all the other actions of insulin (e.g., as a growth factor), effects on neuropeptide secretion, and action on smooth muscle, endothelium, platelet, and erythrocyte function. Figure 1 illustrates the targeting by insulin of four cellular systems not normally considered to be part of the metabolic syndrome. In this article and a subsequent article (8a), we examine the hypothesis that platelet and neurovascular dysfunction are integral parts of the metabolic syndrome and coexist with features of the syndrome,

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Abbreviations: CVD, cardiovascular disease; G_i, G-protein inhibitory; GP, glycoprotein; G-protein, GTP-binding protein; IR, insulin resistance; NO, nitric oxide; PAI, plasminogen activator inhibitor; PGI_2, prostacyclin; PI, phosphoinositide; PLC, phospholipase C; TPA, tissue plasminogen activator; TxA_2, thromboxane A_2.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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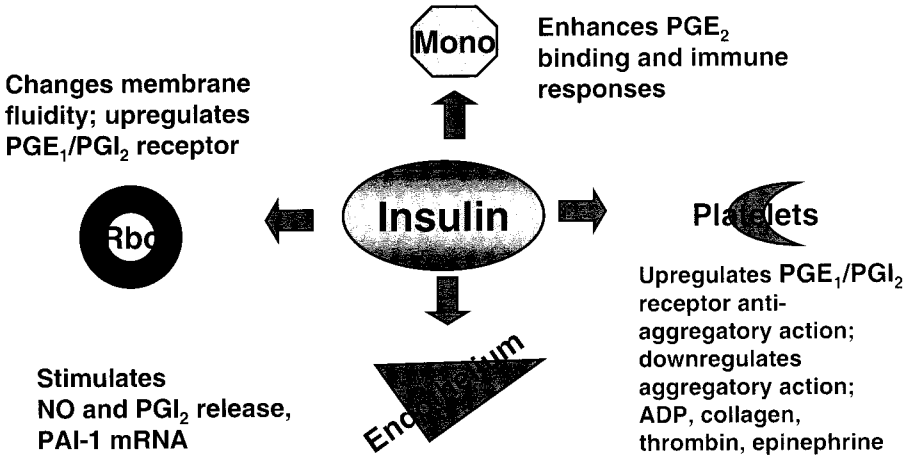

Figure 1—*The effects of insulin on four cellular systems: endothelial cells, platelets, monocytes (Mono), and erythrocytes (Rbc). Insulin is shown to enhance red blood cell membrane deformability, allowing their passage through capillary beds. Insulin exerts endothelial effects by enhancing NO and prostacyclin production, which acts on smooth muscle to cause vasodilation and, in platelets, to inhibit adhesion and aggregation. Insulin also inhibits the production of PAI-1. There are receptors on monocytes for insulin and insulin may exert anti-inflammatory effects. Insulin's action on platelets is to sensitize platelets to the inhibitory actions of prostacyclin and NO on aggregation and to reduce the proaggregatory properties of a number of agonists. PGE_1, prostaglandin E_1; PGE_2, prostaglandin E_2.*

which include IR, dyslipidemia, obesity, and hypertension. The first section of this article focuses on platelet dysfunction.

HOW INSULIN RESISTANCE AFFECTS THE PROCOAGULANT STATE

— IR cosegregates with abnormalities in factors involved in coagulation, including platelet aggregability, platelet adhesion, and levels of thromboxane, von Willebrand factor, factor VIII, tissue plasminogen activator (TPA), and fibrinogen. It decreases fibrinolytic activity due to increased levels of PAI-1. Levels of plasma insulin, proinsulin, cytokines, and glucose and the concentration of modified lipoproteins all affect PAI-1 release (9,10).

The European Concerted Action on Thrombosis Study investigated the pathogenetic and possibly predictive role of the hemostatic system in the progress of coronary heart disease (9). It confirmed the link between hyperinsulinemia and other components of the IR syndrome. In 1,500 angina patients, increased levels of fibrinogen, PAI-1, von Willebrand factor TPA, and prolonged euglobulin clot lysis time correlated with higher circulating insulin levels (from 9 to 12 μU/ml). The strongest relations of insulin with hemostatic factors were observed with fibrinolytic factors, particularly PAI-1 levels, which were substantially higher in patients with higher insulin concentrations ($r = 0.44$, $P < 0.0001$). This relationship diminished somewhat after adjustment for markers of the IR syndrome (mainly BMI and triglycerides) but not after adjustment for markers of inflammation (9). (Inflammation and oxidative stress are involved in enhanced fibrinogen synthesis and atherogenesis [10].)

High levels of PAI-1 have been consistently associated with increased insulin concentrations and decreased insulin sensitivity; increased levels may be a link between IR and coronary heart disease (10). In the Insulin Resistance Atherosclerosis Study, a strong independent correlation between PAI-1 and the insulin precursors proinsulin and split proinsulin was found consistently across different states of glucose tolerance (10). In addition, fasting proinsulin-split products but not insulin correlated with fibrinogen levels. Thus, two crucial factors in the pathogenesis of plaque formation (fibrinogen and PAI-1) relate to levels of circulating insulin or its precursors (10). These findings support the suggestion that fibrinogen may be a risk factor for cardiovascular disease (CVD) (11). However, central to fatal events in atherosclerosis is plaque rupture and adherence of platelets.

PLATELET MECHANISM OF ACTION

— Platelets are small anucleate discoid cells that circulate in the bloodstream and participate in hemostasis (12). Their main function is to plug holes in blood vessel walls. Platelets do this by undergoing a change in shape, adhering to subendothelial surfaces, secreting the contents of intracellular organelles, and aggregating to form a thrombus in response to stimuli generated in endothelia of damaged blood vessels. These proaggregatory stimuli include thrombin, collagen, and epinephrine (which are exogenous to the platelet) and agents such as ADP, which is secreted from platelet storage granules, and thromboxane A_2 (TxA_2), which is synthesized by the platelets during activation (12).

Proaggregatory action

During aggregation, platelets secrete components of the blood coagulation pathway and growth factors necessary for wound healing. Activation of platelets also results in changes in the level of expression of surface glycoproteins (GP) (both integrins and nonintegrins), which act as receptors for platelet agonists and for adhesive proteins involved in platelet aggregation (12).

After platelet activation, P-selectin (also known as GMP-140) translocates from the membrane of α-granules to the plasma membrane (13), the GPIIb-IIIa complex on the plasma membrane undergoes a conformational change that exposes a fibrinogen binding site (14), thrombospondin binding to GPIV increases (15), and thrombin downregulates the von Willebrand factor–binding site on the GPIb-IX complex (Fig. 2) (16,17). Detection of these activation-dependent platelet surface changes by specific antibodies has been used as sensitive assays of platelet activation in whole blood (18).

Antiaggregatory activity

Two of the most studied antiaggregants are the eicosanoid, prostacyclin (PGI_2), and the endothelium-derived relaxing factor nitric oxide (NO). These are released by intact vascular endothelium and antagonize the effects of proaggregants so that thrombi do not form in healthy segments of blood vessels (18). Unlike most proaggregants and antiaggregants that exert their effects by binding to specific

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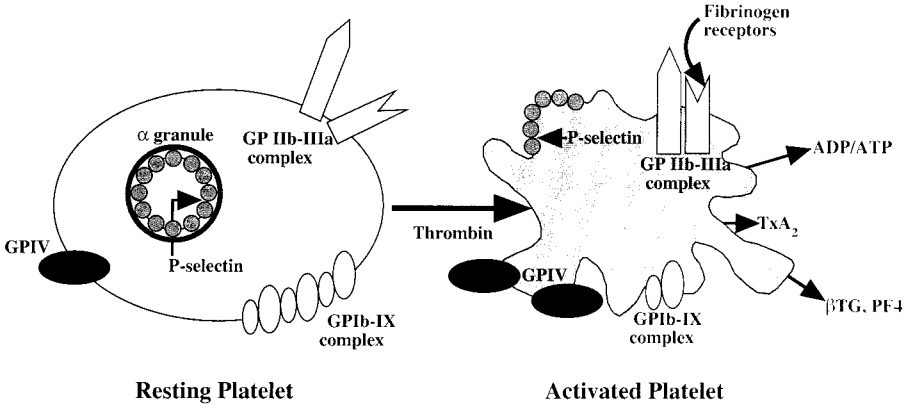

Figure 2—*Surface markers of platelet activation. Changes in the number of GP receptors on the platelet surface occur after activation. Expression on the external platelet surface of GPIIB-IIIa, GPIV, and GMP-140 (P-selectin) increases with activation, whereas that of GPIb-IX decreases. Adapted from Kestin et al. (17).*

receptors on the platelet surface, NO traverses the membrane and directly activates guanylate cyclase. The signals emitted by the activated receptors are transmitted to the interior of the platelet via a distinct number of signal transduction mechanisms, each involving GTP-binding proteins (G-proteins) (12).

The effect of stimulation of the proaggregatory signal transduction mechanisms is the activation of effector systems, such as phospholipase C (PLC)-induced hydrolysis of inositol phospholipids and opening of ion channels. The effector systems of the antiaggregatory mechanisms activate adenylate and guanylate cyclase. Activation of all these effector systems leads to the different physiological responses by inducing changes in phosphorylation state, enzymatic activity, and structural properties of key platelet proteins (18).

Increased platelet activity and an increased tendency for thrombus formation occur in atherosclerosis, heart disease, hypertension, and diabetes. An evolving concept is that enhanced platelet activity may not only derive from procoagulant activity but from unbridling platelet hyperfunction secondary to loss of the restraining action of the antiaggregatory mechanisms. It appears that central to this loss of containment of the platelet and its interaction with vessel endothelium is the resistance to the inhibitory action of insulin coupled with defective endothelial production of the antiaggregants NO and PGI_2.

Abnormalities may occur in potentially all of the mechanisms regulating platelet function discussed above involving platelet-agonist interaction, platelet–vessel wall interaction, platelet-platelet interaction, platelet secretion, and platelet–coagulant protein interaction.

DIABETES, PLATELET ACTIVITY, AND ATHEROSCLEROSIS

— The pathogenesis of atherosclerosis in diabetes has several potential contributors, which include increased intravascular thrombin generation and reduced fibrinolytic potential (19). Fibrinogen levels may also be elevated in diabetes (20), which would contribute to fibrin clot formation and platelet aggregation. Fibrinolytic activity has been reported to be low in type 2 diabetes (21). This is thought to be due to high levels of PAI-1, which inhibit the formation of fibrinolytic plasmin from plasminogen.

PAI levels are strongly correlated with BMI and fasting plasma insulin levels in type 2 diabetes and also with triglyceride levels in nondiabetic obese subjects (21). Furthermore, PAI-1 levels decrease and fibrinolytic activity improves when IR and hyperinsulinemia are reduced by weight loss (22). The elevated PAI-1 levels in subjects with type 2 diabetes may therefore be explained by the IR of these individuals. This may also explain why vascular complications are more common in obese rather than nonobese type 2 diabetic patients.

Endothelial injury or plaque rupture with platelet adhesion and aggregation at the site of injury may be the critical event in producing morbidity and mortality from atherogenesis because most coronary events occur with less than one-third narrowing of the vessel lumen (23). Platelets may therefore assume an important role in the signal event in atherosclerosis in diabetes. This thesis is substantiated by the results of studies in which antiplatelet drugs such as aspirin and dipyridamole protected against stroke and myocardial infarction in both diabetic

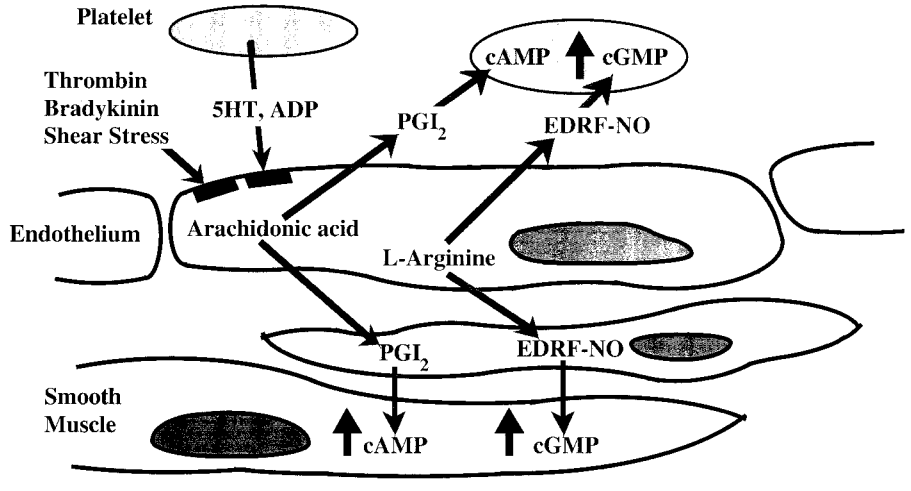

Figure 3—*Synthesis and actions of PGI_2 and NO in blood vessels. Stimulation of endothelial cell receptors by platelet-derived 5-HT (serotonin) or ADP or by thrombin, bradykinin, or shear stress leads to the release of the vasodilator/antiaggregants. Prostacyclin relaxes smooth muscle and inhibits platelet aggregation via the cAMP pathway. Endothelium-derived relaxing factor (EDRF) or NO also relaxes smooth muscle and inhibits platelet aggregation and adhesion via the cGMP pathway. Adapted from Vane et al. (25).*

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Table 1—*Platelet function in type 1 and type 2 diabetes and IR*

Platelet response	IR	Type 1 diabetes	Type 2 diabetes
Antiaggregatory activity of insulin (human platelets)	↓ (42, 62)		↓ (42)
Antiaggregatory activity of NO (human platelets)	↓ (43)		↓ (43)
Antiaggregatory activity of prostacyclin (human platelets)	↓ (62)	↓ (63)	
cGMP response to NO (human platelets)	↓ (44)	↓ (45)	↓ (44, 49)
cNOS activity (human platelets)		↓ (46, 47)	↓ (46, 47)
iNOS activity (human platelets)		↑ (48)	↑ (48)
Prostacyclin binding (human platelets)		→ (38)	→ (38)
Antiaggregatory activity of HDL (streptozotocin rat model)		↓ (50)	
Thrombin-induced PI hydrolysis (human platelets)		↓ (41)	↑ (52–54)
Thrombin-induced PI hydrolysis (streptozotocin rat model)		↑ (51)	
Thromboxane synthesis (human platelets)		↑ (55)	↑ (57)
Thromboxane receptor sensitivity (human platelets)		↑ (56)	
Arachidonic acid uptake (human platelets and streptozotocin rat model)		↑ (58)	
Aggregation in response to LDL (human platelets)		↑ (59, 61)	
Release of PAI-1 (human platelets)			↑ (60)

↓, Decreased; ↑, increased; →, no change. Reference numbers are given in parentheses. NOS, constitutive nitric oxide synthetase; iNOS, inducible NOS.

and nondiabetic individuals and also protected against diabetic retinopathy (24).

PLATELET-ENDOTHELIAL CELL INTERACTIONS

— In healthy vessels, PGI_2 and NO combine to prevent platelet adherence to endothelium and platelet aggregation (18). These antiaggregants are released continually by healthy endothelium, but their synthesis is increased in the vicinity of aggregating platelets in response to plasma thrombin and bradykinin and to platelet-released serotonin, platelet-derived growth factor, interleukin-1, and ADP (Fig. 3) (25). This negative feedback mechanism is thought to limit the growth of the platelet plug to the area of vessel damage and de-endothelialization.

Prostacyclin binds to a specific platelet receptor of the classic seven-transmembrane domain structure linked to a G-protein stimulatory for adenylate cyclase. A G-protein inhibitory (G_i) for adenylate cyclase is linked to the α_2-adrenergic receptor, which binds to epinephrine (26, 27). NO, a much smaller shorter-lived molecule, diffuses across the platelet membrane and directly activates guanylate cyclase. These inhibitory pathways culminate in phosphorylation by cAMP-dependent and cGMP-dependent protein kinases, respectively, and subsequent inactivation of platelet proteins crucial for aggregation (18,28).

There have been reports of decreased vascular synthesis of PGI_2 (29–31) and of decreased synthesis and release of NO in models of diabetes (32,33). Studies in animals have indicated that metabolic control with insulin or pancreatic islet transplantation return vascular PGI_2 synthesis to normal (28). Clearly, impaired synthesis and release of antiaggregants would result in increased platelet activity and response to proaggregants, but the same effect would result if platelets were less sensitive to antiaggregants in diabetes.

Platelets have been shown to respond to PGI_2 and NO to a lesser degree in patients with vascular disease caused by diabetes or other factors. Platelets from diabetic subjects have been reported to have diminished sensitivity to PGI_2 (34) and NO (35). Reduced sensitivity of coronary vascular smooth muscle to NO has been suggested in acute ischemic heart disease (36), indicating that insensitivity to NO may be a defect common to vascular diseases.

Insulin and platelet/endothelial function

A variety of mechanisms may be responsible for an observed decrease in sensitivity to PGI_2 and NO. First, PGI_2 receptor activity could be impaired in diabetes. Although a decrease in receptor number has not been described in diabetes, it has been in heart disease (33), a condition in which receptor number was returned to normal by administration of insulin (35,37).

Modesti et al. (38) reported that platelets from individuals with both type 1 and type 2 diabetes have normal PGI_2 receptor numbers. Therefore, the defect is likely to be downstream from the receptor. Altered responses to PGI_2 may also be manifested through G-protein malfunction, which occurs downstream from the receptor. Livingstone et al. (39) found that the level of G_i in the membranes of platelets from type 2 diabetic patients exhibits a decrease. This decrease correlated with a decreased stimulation of adenylate cyclase in response to activation of the PGI_2 receptor by prostaglandin E_1. Bono et al. (40) studied 10 nondiabetic lean subjects and 10 obese insulin-resistant subjects and found that insulin (100 μU/ml) enhanced cAMP responses to PGI_2 in platelets in lean nondiabetic subjects but not in obese insulin-resistant subjects. However, the mechanism of this impaired cAMP response remains to be determined.

The notion that diabetes affects the activity and expression of G-proteins is not novel. There are other reports in the literature that suggest changes in G-proteins in diabetes. Bastyr et al. (41) demonstrated that GTP-stimulated, but not basal, platelet PLC activity is decreased in type 1 diabetes and that alterations in the subcellular distribution of low–molecular weight G-proteins in type 1 diabetes are correlated with increased aggregation in response to thrombin (37). The mechanisms of platelet dysfunction in type 1 insulin-sensitive and type 2 insulin-resistant diabetic subjects may be quite different. Table 1 compares and contrasts the differences in platelet responses between type 1 and type 2 diabetes.

Kahn et al. (63) reported that there is

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overexpression of a *ras*-related G-protein in skeletal muscle in type 2 diabetes. Work from our laboratory indicates that there is a 10-fold increase in the translocation of rap1B (a G-protein) to the cytoskeleton in activated platelets from patients with type 2 diabetes in the absence of a concomitant increase in the expression of rap1B (64). The significance of the differences in expression, activity, and cellular localization of G-proteins in diabetes has not been determined. In type 2 diabetes, it appears that there may be genetic anomalies in the activity of G-proteins caused by differences in either the level of expression or sequence of genes. Mutations in the G-protein genes themselves may account for the changes in cellular distribution or, alternatively, these changes may be brought about by malfunction of the machinery that carries out posttranslational processing of G-proteins in diabetes (65). It is quite possible that in a disease with a genetic basis, such as diabetes, there is defective posttranslational processing of proteins, which leads to inappropriate activity or subcellular localization of the proteins (37).

Apart from the work of Livingstone et al. (39), little is known about the activities of adenylate and guanylate cyclases in diabetes. However, evidence exists of increased cGMP-phosphodiesterase activity in experimental and human diabetes (66), which may explain abnormalities in sensitivity to antiaggregants in the absence of other defects. Further work needs to be done to elucidate whether there are inherent abnormalities in the specific cyclic nucleotide–dependent protein kinase enzymes because altered activity of these proteins would clearly affect the way platelets respond to antiaggregants.

The endothelium may also contribute to platelet activation in diabetes by releasing von Willebrand factor, a GP constituent of the factor VIII complex, which promotes platelet clumping by binding to the platelet GPIb-IX and IIb-IIIa complexes. Studies have shown elevated levels of von Willebrand factor activity and antigen in plasma of diabetic subjects (67). It is thought that the increased release of von Willebrand factor in diabetes is an indicator of endothelial damage. Insulin can reduce the elevated von Willebrand factor level in experimental diabetes (68), which may provide an explanation for a beneficial effect of insulin in reducing platelet activity.

INTRINSIC ABNORMALITIES IN THE PLATELET

— There is an ever-expanding pool of literature on the enhanced platelet sensitivity to a variety of aggregating agents in vitro, including epinephrine ADP, thrombin, and collagen. It is not clear at this time whether the platelet abnormalities are intrinsic to the platelet or are a consequence of circulating factors that affect platelet function, as has been demonstrated for insulin immunocomplexes (66). It has been reported that the response to epinephrine, ADP, and thrombin are enhanced in human diabetes (29,62,69,70), although it has been difficult to consistently demonstrate hyperaggregatory responses in moderately well-controlled patients with type 2 diabetes (71,72).

The hyperaggregability of platelets in patients with diabetes appears to be independent of the ADP and arachidonate pathways (69,70,73) and is not diminished after insulinization of patients with diabetes for 7 days. This action normalizes blood glucose but does not return the lipid profile to normal (67,74). Colwell et al. (74) hypothesized that in diabetes, a vicious cycle may be set up in which vascular disease may lead to platelet damage, and altered platelet function may contribute to vascular disease. Therefore, it seems that perturbations in the extensive network of cross-talk between tissues may be a factor in the pathogenesis of diabetic vascular complications, although evidence exists of an intrinsic abnormality in platelets in diabetes.

Much of the early work concentrated on the role of the arachidonic acid pathway in the enhanced aggregation of platelets in diabetes. Sagel et al. (73) found that inhibition of cyclo-oxygenase significantly decreased the effect of diabetes on platelets. This was attributed to the increased synthesis of prostaglandin E_2 and TxA_2 in activated platelets obtained from subjects with diabetes (56,75–78). Although platelets from subjects with diabetes were less sensitive to inhibition of the synthesis and action of TxA_2 (75), TxA_2 synthesis does not necessarily correlate with platelet aggregation (56). This is consistent with the evidence that the enhanced platelet aggregation is multifactorial.

As discussed above, the increase in platelet aggregability may not be caused solely by the onset of vascular disease. In an animal model of diabetes, enhanced platelet aggregation and TxA_2 synthesis was detected within days of making rats diabetic with streptozotocin, before vascular disease was evident (30). Also, vascular disease is promoted by platelet activation and release of mitogens, which stimulate vascular smooth muscle cell proliferation, suggesting that platelets play a role in the etiology of atherosclerosis.

ALTERNATE MECHANISMS FOR ENHANCED PLATELET SENSITIVITY TO AGONISTS

— Data such as these highlight the difficulties in relating findings made in vitro with the situation in vivo. It is therefore important to investigate platelet behavior in vivo in the different models of diabetes. Previous studies established along these lines indicate that there is increased platelet turnover in vivo in diabetes, suggestive of increased platelet aggregation in the circulation (67,78).

Inositol phospholipid turnover and calcium release

After the burst of interest in platelet TxA_2 production in the late 1970s and early 1980s had waned, researchers began to focus on possible alternate mechanisms to explain the enhanced platelet sensitivity to agonists in diabetes. The discovery that inositol phospholipid turnover and calcium release were early events in the platelet response (preceding secretion and eicosanoid synthesis) focused attention in that area (79). Suppressed free intracellular magnesium levels have been demonstrated to be an important predictor of platelet thrombosis (80), which correlates significantly with blood glucose, total cholesterol, apolipoprotein B (81), hypertension, IR, and cardiac hypertrophy (82). Exaggerated intracellular calcium and suppressed intracellular magnesium may be associated with the enhanced platelet aggregation associated with type 2 diabetes (83). Most proaggregants, including thrombin, collagen, ADP and TxA_2, initiate platelet aggregation by binding to specific G-protein–linked receptors and activating PLC-mediated hydrolysis of phosphoinositide P_2 (84), which results in release of the calcium-elevating messenger inositol triphosphate

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and the protein kinase C stimulator diacylglycerol (12).

Although efforts have been made to establish an effect of diabetes on platelet phosphoinositide (PI) turnover, a uniform trend has not appeared. For example, PI turnover was shown to be elevated in hyperaggregating platelets in type 2 diabetes (84) but decreased in type 1 diabetes (41). It would seem that enhanced sensitivity to primary agonists of platelet aggregation would be accompanied by an increase in PI turnover; however, because of the multifactorial nature of diabetic platelet hyperaggregability, decreased activity of the PI cycle in type 1 diabetes may represent a compensatory response of platelets to increased activity of another pathway. Alternatively, much of the discrepancy in these studies could be due to case selection and definition of hyperaggregability. To compound the issue, the increase in platelet activity observed with aging has been associated with increased PI turnover (41). This is compatible with the hypothesis that increased platelet activity is associated with increased PI turnover (85).

It has been shown that resting platelets from type 1 diabetic patients with poor metabolic control (HbA_{1c} >8%) had higher calcium content than those from control subjects, whereas thrombin-induced calcium levels were augmented only in patients with good metabolic control who were free from complications (86). As mentioned above, these data are consistent with the report of decreased thrombin-induced PI pathway activity in hyperaggregating platelets in type 1 diabetes (41) and fit in with the compensatory response theory. Basal and collagen-stimulated calcium levels were higher in platelets of type 2 diabetic subjects compared with control subjects (87), which is consistent with the PI data in experiments with type 2 platelets discussed previously in this article. Interestingly, the changes in type 1 diabetes in platelet calcium and aggregation were not affected by acute alterations in in vitro glucose concentration, indicating that glucose does not affect platelet aggregation directly.

Products of advanced glycosylation/ platelet membrane fluidity

Products of advanced glycosylation, the terminal adducts of the nonenzymatic reaction between glucose and the amino groups of protein, accumulate in tissues at an accelerated rate in diabetes (88). The increased extent of glycosylation of platelet membrane proteins in diabetes appears to be related to reduced membrane fluidity (89), which modulates cell function, possibly through alterations in receptor availability. The reduced membrane fluidity of platelets in diabetes may contribute to platelet hyperfunction. Also, enhanced glycosylation of subendothelial proteins may quench NO produced by the endothelium (32) and contribute to reduced platelet inhibition.

Platelet membrane fluidity can also be affected by the plasma lipoprotein profile, which is altered in diabetes (90). Moreover, the increased glycosylation of LDLs in type 1 diabetes has been shown to be responsible for enhanced platelet sensitivity to the aggregating agents thrombin, collagen, and ADP. Watanabe et al. (91) reported that LDLs isolated from type 1 diabetic patients were taken up by platelets and underwent enhanced aggregation to a greater extent than LDLs isolated from matched control subjects. These properties of LDLs from type 1 diabetic subjects were correlated with the degree of glycosylation only. There was no difference in lipid composition of LDLs isolated from diabetic and control subjects and no change in platelet lipid composition. These results suggest a mechanism for the altered membrane fluidity and platelet hyperaggregability in diabetes. In both type 1 and type 2 diabetes, the prothrombotic tendency, which includes reduced endothelial cell production of prostacyclin and activators of fibrinolysis, together with increased platelet reactivity, is associated with increased oxidative stress and lipid peroxidation due to excess free radical activity (92,93).

Oxidized LDL, for example, has been shown to reduce the antiaggregatory properties of endothelial NO in vitro (94). Thus, it is not only the level of the lipoprotein but its glycated and oxidized state that may have relevance in the procoagulatory state.

Increased GP expression

Evidence also exists for increased GP expression on the platelet surface in diabetes. Increased numbers of GPIb and GPIIb-IIIa complexes are present on platelets in both type 1 and type 2 diabetes (95). GPIb acts as a receptor for von Willebrand factor, to which platelets are exposed at injury sites, and acts as a substrate for thrombin. GPIIb-IIIa acts as a receptor for several adhesive proteins, including fibrinogen, which are involved in aggregation. Increases in GP expression on platelets may therefore contribute to platelet hypersensitivity of diabetes.

It is probable that the etiology of platelet hyperaggregability differs in the two major forms of diabetes and in other instances of vascular complications. It remains to be seen whether the relatively novel PI 3-kinase pathway, which appears to be involved in platelet secretion and aggregation, is altered in diabetes (96). Even if it is, the challenge will be to demonstrate whether it is the cause or effect of the underlying tendency of platelets to hyperaggregate.

INSULIN MAY HAVE DIRECT AND INDIRECT ACTIONS ON PLATELETS

— The hypothesis that platelets behave differently in type 1 and type 2 diabetes leads to a discussion of the role of IR in platelet hyperaggregability in diabetes. Platelets have been shown to be targets of insulin action because they retain a functional insulin receptor capable of insulin binding and autophosphorylation (97). Insulin is generally thought to reduce platelet responses to the agonists ADP, collagen, thrombin, arachidonate, and platelet-activating factor (7). A clue to this action of insulin is the finding that insulin downregulates the number of α_2-adrenergic receptors on platelets (41,98). Because epinephrine potentiates the effects of other aggregating agents (99) and stimulates G_i-mediated inhibition of adenylate cyclase (26,100), it is clear that an effect of insulin to modify the action of epinephrine would attenuate platelet responses to the other aggregants. Udvardy et al. (101) reported decreased platelet insulin receptor number and affinity in subjects with type 2 diabetes, which suggests that reduced insulin sensitivity may account for platelet hyperactivity in type 2 diabetes. Moreover, researchers showed that in nondiabetic patients with acute ischemic heart disease, there is decreased binding of insulin and PGI_2 to platelets (102). These deficiencies are transient and improve after recuperation. Other studies showed that the impaired responses of platelets to PGI_2 in coronary heart disease are normalized by physiological quantities of insulin administered both in vitro (34,103) and in vivo (40,104). The effect

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of insulin was to increase the number of PGI_2 binding sites on platelets, which increased the cAMP response to PGI_2. This effect was observed in platelets from acute ischemic heart disease patients and healthy control subjects and was more than twofold greater (34,103,105). These studies indicate the potential importance of insulin in maintaining normal platelet sensitivity to PGI_2 and suggest a possible mechanism whereby platelets are more active in diabetes. There appear to be no data in the literature on the effects of insulin on PGI_2 responsiveness of platelets from subjects with diabetes, although this is obviously an important issue. We studied the effects of insulin on platelets of obese insulin-resistant patients compared with lean healthy control subjects (40). Insulin impaired the inhibition of platelet aggregation in response to proaggregatory agents in obese subjects compared with lean subjects. The resistance of platelets in individuals with diabetes to the inhibitory actions of insulin and PGI_2 is borne out by the relative resistance of people with diabetes to reduce CVD, as shown in a large multicenter study (106).

EFFECTS OF INSULIN SENSITIZATION ON THE PROTHROMBOTIC STATE

— Troglitazone, like vitamin E, has been shown to have potent inhibitory effects on human platelet aggregation via suppression of thrombin-induced activation of PI signaling in platelets (107). This effect was not, however, reproducible with pioglitazone and may therefore be due to troglitazone's unique structure, which includes an α-tocopherol moiety. In addition, troglitazone treatment in doses of 200–400 mg/day as monotherapy or in combination with insulin has been reported to significantly lower PAI-1 levels (108).

SUMMARY: PLATELET DYSFUNCTION IN DIABETES

— In vitro studies have shown a number of anomalies in the mechanisms of action of platelets in diabetic subjects. These anomalies account for hypersensitivity of platelets to aggregants and hyposensitivity to antiaggregants and are thought to contribute to enhanced atherosclerosis via increased platelet activity at sites of vessel injury. Changes in platelets in diabetes include enhanced GP receptor binding of agonists and adhesive proteins; decreased membrane fluidity; enhanced activation of the arachidonic acid pathway resulting in increased TxA_2 formation; altered PI turnover leading to changes in diacylglycerol and inositol triphosphate production, calcium mobilization, and protein phosphorylation; impaired responses to antiaggregants resulting in decreased PGI_2 receptor binding, cyclic nucleotide production and cyclic nucleotide–dependent protein phosphorylation; and reduced sensitivity to the inhibitory actions of insulin. These changes translate to impaired PGI_2 stimulation of cAMP and blindness to the inhibitory actions of both PGI_2 and NO. Platelet dysfunction coupled with decreased endothelial production of these antiaggregatory agents conspire to amplify the risk of CVD in patients with type 2 diabetes.

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