Abnormalities of Platelet Function in Hypertension and Diabetes

Pavel Hamet, Romana Skuherska, Stephen C. Pang, and Johanne Tremblay

SUMMARY The increased frequency of hypertension in diabetes and of abnormalities of carbohydrate metabolism in hypertension are now well established. It is conceivable that the high coincidence of the two diseases is based on a common metabolic defect. Studies of platelets permit the evaluation of the stimulatory, phosphoinositol-linked and the inhibitory, cyclic adenosine 3',5'-monophosphate-dependent pathways of cell activation. Furthermore, platelets may be relevant for the development of angiopathy through their contents of growth factors. Abnormalities of platelet aggregation have been demonstrated in hypertension and diabetes. They are accompanied by exaggerated stimulation of adenylyl cyclase in hypertension and abnormal activity of cyclic guanosine 3',5'-monophosphate phosphodiesterase in diabetes. Defective function of platelets is also observed in patients and animals when the two diseases are present at the same time. Both increased and decreased aggregation have been described in these two diseases in the literature. The apparent discrepancies may be due to different types of platelet preparation, evaluation of aggregation, evolution of defect with age, and form of the disease. Integrated studies of biochemical mechanisms responsible for cell activation are needed to characterize the exact defect present in diabetes and hypertension in platelets. (Hypertension 7 [Suppl II]: H-135-II-142, 1985)

KEY WORDS  • platelet aggregation  • rat  • human

Hypertension and diabetes are among the most significant risk factors in cardiovascular disease. As in other countries, hypertension in Canada is present in about 50% of diabetics and the rate of occurrence of diabetes is high among hypertensives (Table 1). In our own clinics at the Clinical Research Institute of Montreal and Hotel-Dieu Hospital, we have noted that 43% of diabetics have increased blood pressure and 31% of hypertensives have abnormal blood glucose levels.1

As we suggested elsewhere,2 the pathogenesis and coexistence of both these important risk factors must be evaluated on two fronts. Their coexistence is partly a complication of diabetes; thus, tissue damage, microangiopathy and macroangiopathy, as well as nephropathy can at least partially explain the increased occurrence of hypertension in diabetes. On the other hand, the enhanced coincidence of these two diseases may be due to common genetic and nutritional factors, enzymatic abnormalities in the heart and vessels, and other anomalies that may be shared at the level of blood pressure control and glucoregulation.

This review attempts to pinpoint platelet abnormalities present in both diseases as they may reflect defects in the transmission of extracellular to intracellular signals with their potential contribution to the development of vascular disease in diabetes and hypertension.

Relevance of Platelets in the Study of Diabetes and Hypertension

Platelets are particularly suitable tissues for investigative purposes since they can be isolated as a single cell type from both animal and human sources, and simultaneous studies of their function, aggregation, and biochemical mechanism(s) responsible for their activation can be performed in vitro. In addition, platelets share several common pathways with vascular smooth muscles; hence, parallels are observed between aggregation and contraction, and between inhibition of aggregation and relaxation. In the last few years, the mechanisms leading to positive (contraction, aggregation) and negative (relaxation, antiaggregation) actions have been significantly elucidated. Several groups3-6 have enriched our understanding of the positive stimulatory pathway. The negative path-
Platelets are also involved in homeostatic repair of the vascular wall. At present, there are at least three known types of platelet growth factors: 1) platelet-derived growth factor (PDGF), a cationic peptide that is capable of enhancing, among other cells, the growth of vascular smooth muscle; 2) anionic growth factor, which appears to be identical to epidermal growth factor; and 3) transforming growth factors. In addition to their involvement in the maintenance of vessel integrity, these factors may, in pathological situations, be responsible for abnormal proliferation of cellular components of the vessel wall and thus be relevant to the evolution of microangiopathic and macroangiopathic complications in hypertension and diabetes.

Platelet Aggregation in Hypertension and Diabetes

It was demonstrated in both hypertension and diabetes that platelets undergo functional anomalies of aggregation and secretion. These changes may 1) reflect their abnormal responsiveness to aggregational and inhibitory stimuli and 2) provoke, as a consequence, the release of their contents, including growth factors. As discussed later, it is nevertheless difficult to establish whether or not platelet aggregation is increased or decreased in hypertension and diabetes, since studies reported in the literature suggest both possibilities.

Platelets in Hypertension

Preliminary experiments by our group illustrated an augmented aggregation of washed platelets in spontaneously hypertensive rats (SHR) in response to a divalent cation, ionophore A23187. Since the extent of this change was modulated by extracellular calcium, it perhaps represents abnormal transport or storage of intracellular calcium in hypertensive platelets.

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Studies by other groups demonstrated an increase of ADP-induced aggregation in prehypertensive patients\(^9\) and a decrease of ADP-elicted aggregation in hypertensive animals.\(^{20, 21}\) As discussed later, it is apparent that the inhibitory and stimulatory pathways are modified differently at various stages of hypertension.

### Platelets in Diabetes

We recorded qualitatively opposite changes in platelets from spontaneously diabetic (BB strain) and streptozotocin-induced diabetic rats.\(^{14}\) In the spontaneously diabetic animals, the decreased aggregation was accompanied by increased activity of cyclic guanosine 3',5'-monophosphate (cGMP) phosphodiesterase, whereas in the streptozotocin-provoked diabetics, contrasting observations were made. These results draw attention to yet another mechanism of the modulation of cellular function, namely, cGMP. Unlike the well-understood scheme of cAMP generation and its effects, cGMP remains a biological enigma. This cyclic nucleotide is generated by its synthesizing enzyme, guanylate cyclase, which is present either as a membrane-bound or soluble heme-dependent enzyme.\(^{22}\) While direct stimulation of the soluble cyclase by nitroso agents such as sodium nitroprusside (SNP) is calcium-independent,\(^{23}\) the activation by hormones is usually calcium-dependent\(^{24}\) and indirect. The calcium-dependent pathway probably involves release from cell membranes of arachidonic acid, which is able to increase guanylate cyclase activity in a way that is mutually exclusive from the nitroso agents.\(^{25}\)

A part of the effect of cGMP may be expressed by way of its protein kinase.\(^{26}\) Nishizuka\(^1\) postulated that the phosphorylation induced by cGMP protein kinase is inhibitory to the positive pathway of C-protein kinase. In platelets, however, the activity of cGMP-dependent protein kinase is undetectable. The only detectable binding protein for cGMP present in platelets is related to a specific type of cGMP-hydrolyzing enzyme, phosphodiesterase.\(^{27}\) We have already mentioned that this cGMP-binding phosphodiesterase can be regulated from the exterior of platelets by aggregational and antiaggregational agents.\(^{28}\) The apparent pathway of this activation includes cAMP-dependent phosphorylation of the enzyme.\(^{29, 30}\) In addition, we recently observed that cGMP-binding phosphodiesterase can also be activated by oxidative mechanisms.\(^{31}\) Since the turnover of cGMP appears to be an extremely rapid phenomenon, Goldberg et al.\(^{32}\) proposed that at least part of the cGMP function is expressed through its hydrolysis rather than through its levels. It could be speculated at present that cGMP hydrolysis is responsible for rapid events, such as ionic movement within cells, leading to a modification of cell function (Figure 2). Since our studies indicated that cGMP hydrolysis is modulated by an oxidative mechanism, it is interesting to note that Watanabe et al.\(^{33}\) detected a decrease of an important reductive agent, tocopherol (vitamin E), in platelets of humans with diabetes. Further investigations are needed to elucidate the involvement of an abnormal regulation of cGMP hydrolysis in platelet aggregation among diabetics.

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**Platelets in Hypertension and Diabetes**

Interaction of Hypertension and Diabetes in Platelet Aggregation

To evaluate the concomitant effects of hypertension and diabetes on platelet function, we induced diabetes by streptozotocin injection in SHR and control Wistar-Kyoto rats (WKY) (Table 2). It is noteworthy that the SHR were significantly more sensitive to streptozotocin: the same dose of this agent induced more severe diabetes in the hypertensive animals. A similar observation was reported by other investigators.\(^{34}\) The reason for the increased sensitivity to the Langerhans' islet beta cell toxin is not known, but studies by Nara et al.\(^{35}\) suggested that SHR have an already increased incidence of glucose intolerance. An inherited defect of beta cell function may therefore be exaggerated by a low dose of streptozotocin. Alternatively, the abnormally high carbohydrate tolerance of SHR may be a reflection of a shared biochemical defect leading to both hypertension and diabetes. Figure 3 demonstrates the responsiveness of platelets from normotensive, hypertensive, and diabetic animals to the divalent cation ionophore A23187 and to a low dose of thrombin and ADP. Whether expressed as a percentage of maximum aggregation (Figure 3A) or as an initial slope of aggregation (Figure 3B), it appears that platelets from both hypertensive and diabetic animals reveal an exaggerated response to thrombin. Initial rates of aggregation also suggest a decrease of responsiveness to ionophore A23187.

In a second study, we evaluated aggregation in washed platelets from human subjects (Table 3). The diabetic patients were controlled by diet only, and the
TABLE 2. Characteristics of Rats in a Study of the Interaction of High Blood Pressure and Diabetes

<table>
<thead>
<tr>
<th>Measurement</th>
<th>WKY controls</th>
<th>WKY-STZ</th>
<th>SHR</th>
<th>SHR-STZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>5</td>
<td>6</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>261 ± 7</td>
<td>248 ± 10</td>
<td>299 ± 7*</td>
<td>265 ± 6</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>124 ± 3</td>
<td>118 ± 4</td>
<td>170 ± 2†</td>
<td>168 ± 2†</td>
</tr>
<tr>
<td>Pulse rate (beat-min⁻¹)</td>
<td>372 ± 22</td>
<td>394 ± 15</td>
<td>420 ± 24</td>
<td>440 ± 13‡</td>
</tr>
<tr>
<td>Blood glucose (mg·dl⁻¹)</td>
<td>154 ± 10</td>
<td>239 ± 28‡</td>
<td>162 ± 14</td>
<td>454 ± 26†</td>
</tr>
</tbody>
</table>

STZ = streptozotocin-induced diabetes.
* p < 0.01.
† p < 0.001.
‡ p < 0.05.

FIGURE 3. Aggregation of albumin gradient-column-washed platelets in control, hypertensive and streptozotocin-induced diabetic rats that were approximately 14 weeks old at the time of sacrifice. Streptozotocin (STZ), injected through the tail vein at a dose of 40 mg/kg, induced glucosuria within 18 to 24 hours. Platelet aggregation was studied on Day 7 after injection. The top panel expresses aggregation as a percentage of maximum optical density. The bottom panel depicts the initial rate of aggregation from the steepest slope.
hypertensive patients had moderate, stable essential hypertension. It is evident from Figure 4 that the platelets from both diabetic and hypertensive subjects showed a decreased aggregation in response to all the agents tested.

Reasons for Divergent Results of Platelet Function

Our results illustrate a divergence of platelet aggregation in human subjects and in animal models. Disparate findings of platelet aggregation are also reported in the literature on both diabetes and hypertension.36,37 Several reasons for these divergences are apparent. The first is perhaps the mode of expression of aggregation in itself. Thus the rather frequently reported increase of aggregation in streptozotocin-induced diabetics usually represents an augmentation of a late phase of aggregation, as demonstrated by Halushka et al.38 This late phase of aggregation perhaps denotes the abnormalities of metabolism of the prostaglandin pathway, whereas the initial rate of aggregation is more a reflection of anomalies in the activator pathway related to phosphoinositol turnover, or in the inhibitory pathway represented by the cAMP system. Various degrees of abnormalities are also observed with different expressions of aggregation. One way to look at aggregation is to record the maximum increase in optical density and to express it as a percentage of aggregation; another way is to compute the initial rate of aggregation from the steepest slope (see Figures 3 and 4).

The type of platelet may be another reason for the divergent results in the literature. Most of the reported studies were performed in platelet-rich plasma. This preparation has the advantage of rapidity and it is perhaps a better reflection of the in vivo situation. On the other hand, platelets in plasma are exposed to humoral circulating factors and thus their own biochemical pathways cannot be evaluated directly. For this reason, we used washed platelets. Our preliminary data were generated from platelets washed by differential centrifugation.13 Since we observed that these platelets exhibit pseudopods and microaggregates, we recently adopted the preparation technique described by Timmons and Hawiger39 using albumin gradient and column gel filtration. Figure 5 illustrates that qualitatively opposite results can be obtained with these two types of preparations. In this experiment, a pool of platelet-rich plasma from three hypertensive and three normotensive animals was separated in two: one-half was washed by centrifugation and the other was purified on albumin gradient and Sepharose 2B (Pharmacia), column. The platelets processed by centrifugation revealed decreased aggregation in response to ADP alone or in combination with epinephrine in SHR, whereas increased aggregation was observed in platelets prepared by column filtration after exposure to these same agents and thrombin. It is conceivable that platelets processed by centrifugation are either preactivated or more contaminated by ADP, thus responding less in hypertensive animals, while the opposite occurs in platelets isolated by gel filtration. Although both types of preparations reveal "abnormalities" in the SHR, their extent and even their direction are quite different.

Furthermore, the age of the patients and stage of disease are not sufficiently underlined in publications on platelet aggregation in hypertension and diabetes. We have observed normal aggregation in response to ADP in young SHR, while decreased aggregation of the same type of platelet preparation has been found in adult SHR (Figure 6). Similarly, hyperaggregability of platelets, described by Nara et al.,19 was demonstrated in the prehypertensive stage. The studies by Tomita et al.,20 which demonstrated hypoaggregability of platelets in stroke-prone SHR (SHRSP), were actually very similar to our own experiments, since these authors detected enhanced aggregation at 4 weeks of age, while aggregation with ADP, thrombin, and collagen was decreased in older animals with established hypertension. It would appear from these investigations that hypoaggregability is possibly a phenomenon that is secondary to hypertension, whereas increased aggregation may be present initially and therefore genetically determined.

Relevance of Platelets to Studies of Growth Abnormalities in Hypertension and Diabetes

It is well known that the vascular smooth muscle is hyperplastic in SHR and in humans. Our own studies revealed hyperplasia of the heart and kidney at birth in SHR40 and an increase of thymidine incorporation in vascular organs in neonates.41 Nevertheless, there is complete lack of research on the role of platelets and their growth factors in these abnormalities. We recently completed a study of patients with insulin-dependent diabetes and observed a significantly increased growth potential in platelet extracts from those with poorly controlled disease.42,43 It may be of clinical significance that this enhanced growth potential in platelets from diabetics is normalized by intensive insulin therapy after a long period of time,44 as is platelet aggregation by an artificial pancreas.45 It is not clear which of the growth factors contained in platelets is responsible for the increased growth potential. Never-
Figure 4. Aggregation of albumin gradient-column-washed platelets in human subjects with diabetes and hypertension. The top panel expresses aggregation as a percentage of maximum optical density. The bottom panel shows aggregation as the steepest slope of the initial rate.

Nevertheless, it is conceivable within the framework of the hypothesis of Ross and Glomset that the augmented growth potential in platelets may be involved in the pathogenesis of vascular smooth muscle cell hyperplasia leading to atherosclerosis. Alternatively, the increased growth potential of platelets may be a reflection of an enhanced platelet turnover with the presence of younger platelets in the circulation of patients with this disease.

**Future Needs**

At the present time, studies are required to determine whether or not platelet defects in diabetes and hypertension are primary or secondary. It will be necessary to establish whether platelets express genetic defects in these diseases or whether they merely mirror metabolic and homeostatic adaptation to these maladies. To accomplish this goal, it is first essential to find the best way to evaluate platelet function in vitro to reflect in vivo situation more reliably.

Further studies of the differential release of platelet growth factors into the circulation and their target effects on several types of vessel wall cells are also required. It is of utmost importance to evaluate the beneficial and/or detrimental actions of antihypertensive and antidiabetic therapy on the platelet growth
PLATELETS IN HYPERTENSION AND DIABETES

PLATELETS IN HYPERTENSION AND DIABETES

PLATELETS WASHED BY CENTRIFUGATION COLUMN

AGGREGATION INDUCED BY ADP (FREE Ca** 3mM)

FIGURE 5. Aggregation in pool samples from three hypertensive SHR and three normotensive WKY. The results are expressed as a percentage of aggregation observed in the normotensive animals. The concentration of ADP was 2 mM and epinephrine 10 μM; ionophore A23187, 0.25 μM; thrombin 0.33 U/ml.

FIGURE 6. Aggregation of washed platelets from three different age groups of SHR and WKY controls in response to various doses of ADP (S. Adnot and P. Hamet, unpublished data, 1983).

potential, as it may be of consequence in the development of macroangiopathy. Finally, integrated studies of various pathways of cellular activation and inhibition, including phosphoinositol turnover, C-protein kinase, and cAMP-dependent protein kinase and their mutual interactions, will be necessary in order to assess the metabolic bases of hypertension and diabetes.

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