Platelet Aggregation in Young Men With Contrasting Predisposition to High Blood Pressure

Mark E.C. Dockrell, Brian R. Walker, Joseph P. Noon, Graham C.M. Watt, Brent C. Williams, and David J. Webb

In essential hypertension, abnormal platelet function may induce vasospasm and predispose to thrombotic vascular occlusion. We studied in vitro aggregability in platelets from young men with contrasting predisposition to hypertension, defined by their own blood pressure and blood pressures of their parents. Among offspring of parents with low blood pressure, higher blood pressure was associated with impaired aggregation in response to epinephrine (2 × 10^{-8} to 5 × 10^{-8} mol/L), which was unaffected by endothelin-1 (10^{-9} mol/L). By contrast, among offspring of parents with high blood pressure, higher blood pressure was associated with normal aggregation to epinephrine and potentiation of the primary phase of aggregation by endothelin-1. We conclude that enhanced platelet sensitivity to endothelin-1 appears to be a feature of the familial predisposition to hypertension, rather than a nonspecific consequence of high blood pressure.


KEY WORDS: Platelet, hypertension, endothelins, catecholamines, epinephrine.

Platelets may have a role in the pathophysiology of essential hypertension; alterations in platelet numbers and function influence their release of vasoactive mediators, which induce local vasospasm, and enhanced aggregation of platelets could contribute to the vascular occlusion that complicates hypertension. In case-control studies, platelets from patients with essential hypertension demonstrate a number of abnormalities, including increased cytosolic calcium concentrations, impaired down-regulation of α2-adrenoceptors, and increased sensitivity to ADP and arachidonic acid. In addition, we and others have described an interaction between endothelin-1 and platelet sensitivity to catecholamines in healthy subjects, which may also be relevant in hypertension. In healthy subjects, endothelin-1 does not induce platelet aggregation directly but in the presence of epinephrine it potentiates the cyclooxygenase-independent primary phase of aggregation and attenuates the secondary

Received April 2, 1998. Accepted August 14, 1998.
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Published by Elsevier Science, Inc.
cyclooxygenase-dependent phase. It is the latter secondary aggregation that is associated with formation of large stable aggregates during episodes of vascular occlusion. In essential hypertension, the effect of endothelin-1 on secondary platelet aggregation is reported to be reduced.

However, case-control studies in patients with hypertension cannot establish whether altered platelet function occurs as a consequence of the higher blood pressure, or whether it could be a factor that contributes to higher blood pressure. To address this issue for other variables associated with hypertension, we have investigated a cohort of young adults with contrasting predisposition to high blood pressure, as defined both by their own blood pressure and the blood pressures of their parents. We have argued that factors that are secondary to hypertension will be present in individuals with higher blood pressure irrespective of parental blood pressures, but factors that are involved in the familial predisposition to hypertension may be present in individuals with higher blood pressure only if their parents have high blood pressure. Examples of the former pattern include the association between insulin resistance and hypertension, whereas the latter pattern applies for indices of microvascular structure, glucocorticoid secretion, and enhanced sympathetic nervous system activity. In the current study of subjects from this cohort, we investigated in vitro platelet aggregation induced by epinephrine in the presence and absence of endothelin-1.

MATERIALS AND METHODS

Selection of Participants From the Four Corners Study

The sampling method has been described in detail elsewhere. Blood pressure was measured in 603 married couples in 1979 and in 864 of their offspring, then aged 16 to 24 years, in 1986. Age-adjusted Z-scores were used to define tertiles for both offspring and mean parental blood pressure. Offspring for whom both their own blood pressure and the mean blood pressure of their parents were outside the middle tertile were identified as belonging to one of “four corners” (Figure 1): OL/PL (offspring blood pressure classified as low, parental blood pressure low); OH/PL (offspring blood pressure high, parental blood pressure low); OL/PH (offspring blood pressure low, parental blood pressure high); or OH/PH (offspring blood pressure high, parental blood pressure high). Subgroups of offspring randomly selected from these groups have participated in previous investigations, as described in recent papers.

Most previous studies of the familial predisposition to hypertension have studied two groups: offspring of hypertensive parents and offspring of normotensive parents. However, the statistical power of this approach is weak, as most offspring of hypertensive parents turn out not to have inherited the hypertensive trait. The four-corners approach provides an additional characterization on the basis of offspring blood pressure, to distinguish offspring who have inherited their parents’ predisposition to high blood pressure (OH/PH) from those who have not inherited the predisposition (OL/PH). Similarly, among the offspring of parents with low blood pressure, the four-corners approach distinguishes those who have high blood pressure for reasons other than inheritance (OH/PL) from those who inherited their parents’ predisposition to low blood pressure (OL/PL). Although there will inevitably be some subjects in whom these predictions are not fulfilled, the predictions for the groups made on the basis of blood pressure in 1986 (Figure 1) have proved to be remarkably robust on the basis of blood pressure measured in 1993 to 1995 (Table 1). No individuals in the study have developed hypertension requiring treatment during this time.

Comparison of subjects from the four groups may elucidate abnormalities that precede the development of high blood pressure (evident when comparing sub-
In most subjects, primary and secondary phases of aggregation are readily distinguished as two phases of decreasing light transmittance. However, the concentrations of epinephrine at which primary and secondary aggregation occur vary substantially between subjects. Endothelin-1 has opposing effects that increase the intensity of primary aggregation and decrease the intensity of secondary aggregation.\(^3\)\(^7\)\(^8\) Results are therefore shown for the total aggregation, comprising both primary and secondary aggregation, at each concentration of epinephrine in the absence of endothelin-1 (Figure 2a). In addition, once threshold concentrations of epinephrine that induced primary and secondary aggregation were identified for each subject, aggregations at these two concentrations were repeated in the presence of endothelin-1 (1 nmol/L) and data are presented for the intensity of primary and secondary aggregation in the presence and absence of endothelin-1 (Figure 2b).

**Statistics** Platelet aggregation in response to epinephrine without endothelin-1 was compared among the four groups by two-way repeated measures analysis of variance (ANOVA). Intensity of primary and secondary aggregation were compared within groups by paired Student’s t tests and among the four groups by single factor ANOVA followed by unpaired Student’s t tests where appropriate. Statistical significance was accepted with a two-way \(P < .05\).

**RESULTS** In the absence of endothelin-1, platelet aggregation in response to epinephrine was different among subjects from the four groups (Figure 2a). In the offspring of parents with low blood pressure, higher blood pressure was associated with lower aggregation across the range of epinephrine concentrations (ie, OH/PL < OL/PL). By contrast, in offspring of parents with high blood pressure, aggregation was intermediate between the other two groups and there was no statistically significant relationship with offspring blood pressure (ie, OH/PH = OL/PH).

Addition of endothelin-1 resulted in enhanced primary aggregation only in offspring of parents with high blood pressure who themselves had high blood pressure. However, the concentrations of epinephrine at which primary and secondary aggregation occur vary substantially between subjects. Endothelin-1 has opposing effects that increase the intensity of primary aggregation and decrease the intensity of secondary aggregation.\(^3\)\(^7\)\(^8\) Results are therefore shown for the total aggregation, comprising both primary and secondary aggregation, at each concentration of epinephrine in the absence of endothelin-1 (Figure 2a). In addition, once threshold concentrations of epinephrine that induced primary and secondary aggregation were identified for each subject, aggregations at these two concentrations were repeated in the presence of endothelin-1 (1 nmol/L) and data are presented for the intensity of primary and secondary aggregation in the presence and absence of endothelin-1 (Figure 2b).

**Platelet Aggregation** The method has been described in detail elsewhere.\(^7\) Platelet rich plasma was prepared by centrifugation at 800 g for 10 min. A 2 mL-aliquot of this plasma was centrifuged for a further 5 min at 3000 g to give platelet-poor plasma. Platelet-rich plasma was stored under 95% O\(_2\)/5% CO\(_2\) for <120 min at room temperature before use.

Aggregation was studied in a Malin six-channel light transmittance aggregometer (Malin Electronics, Dumfries, Scotland). Platelet poor plasma was used to calibrate 100% transmittance and platelet rich plasma used to calibrate 0% transmittance. Aliquots of 890 \(\mu\)L of platelet-rich plasma were incubated at 37°C for 3 min before the addition of 100 \(\mu\)L of physiological saline vehicle or endothelin-1 (1 nmol/L). After 1 min, either 10 \(\mu\)L of saline vehicle or epinephrine (0.02 to 5.0 \(\mu\)mol/L) was added and aggregation recorded for 10 min.

<table>
<thead>
<tr>
<th>Offspring BP</th>
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<th>Low</th>
<th>High</th>
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<td>11</td>
<td>11</td>
<td>11</td>
<td>.17</td>
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<td>23 ± 3</td>
<td>23 ± 3</td>
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</tr>
<tr>
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<td>8</td>
<td>6</td>
<td>9</td>
<td>(\chi^2) (P = .88)</td>
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pressure (ie, OH/PH > OL/PH, but OH/PL = OL/PL) (Figure 2b). There was no measurable effect of endothelin-1 on secondary aggregation in this study.

**DISCUSSION**

This study shows that two aspects of platelet function in vitro are correlates of the predisposition to high blood pressure in young adults. Specifically, aggregation to epinephrine is greater in young men with lower blood pressure if their parents had low blood pressure; this phenomenon is therefore associated with a familial predisposition to low blood pressure later in life. By contrast, endothelin-1 potentiates epinephrine-induced primary aggregation in young men with high blood pressure only if their parents had high blood pressure; this phenomenon is therefore associated with a familial predisposition to high blood pressure later in life.

The differences in platelet responses to epinephrine may reflect differences in other aspects of adrenal medullary function in these subjects. The subjects with enhanced sensitivity come from group OL/PL, in which the risk of subsequent hypertension is low, and we have shown that circulating catecholamine levels and sensitivity to mental stress are lower than in other groups. Reduced catecholamine levels might result in up-regulation of $\alpha_2$-adrenoceptors on platelets. The impact of this difference in sensitivity to epinephrine on platelet function in vivo is difficult to estimate. It is most likely to be important under conditions of stress, such as during a myocardial infarction, when epinephrine concentrations are substantially elevated.

The current study also confirms our previous observation that endothelin-1 potentiates epinephrine-induced primary aggregation in platelets. However, this effect is restricted to a subgroup of subjects (OH/PH in this study), which may explain the difficulty that others have had in replicating the effects of endothelin-1 using less selective sampling strategies. Although there were differences in epinephrine concentrations used to induce primary and secondary platelet aggregation in different groups, the intensity of primary aggregation induced was very similar in the absence of endothelin-1. Moreover, the pattern of differences in sensitivity between groups was not the same for endothelin-1 and epinephrine, so that the choice and concentration of primary agonist does not explain the observed difference in sensitivity to endothelin-1.

Although there was a trend for endothelin-1 to reduce the intensity of secondary aggregation when all subjects were included in the analysis (data not shown), relatively few subjects exhibited secondary aggregation and so statistical power was inadequate to detect the effect of endothelin-1 or any difference among the four groups.

With respect to primary aggregation, the difference in platelet responses to endothelin-1 between groups...
is intriguing. We have shown that venoconstriction to endothelin-1 in vivo is enhanced in patients with essential hypertension,\textsuperscript{15} an effect that is most likely to be mediated by enhanced activation of ET-A receptors on vascular smooth muscle.\textsuperscript{16} The current data suggest that enhanced sensitivity to endothelin-1 also occurs in platelets, where its effects may also be mediated by ET-A receptors.\textsuperscript{17} Moreover, the observation that enhanced sensitivity to endothelin-1 is restricted to young adults with higher blood pressure only if their parents have high blood pressure suggests that this is not a nonspecific consequence of higher blood pressure per se, but may be part of the familial predisposition to hypertension. This result reinforces the endothelin receptors, particularly the ET-A receptor, as candidate genes to explain the pathophysiology of hypertension and the endothelin system as a potential therapeutic target in hypertension.

REFERENCES