Platelet aggregation studies during transient hypoglycaemia

A potential method for evaluating platelet function

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SUMMARY The effect of acute hypoglycaemia on platelet function was examined in patients undergoing an insulin stress test. Enhanced platelet aggregation was observed in all cases but platelet count, platelet adenine nucleotides, and the plasma level of von Willebrand factor were unchanged overall.

The onset of the hypoglycaemia-induced increase in platelet aggregation coincided with the lowest blood glucose levels recorded and with the clinical signs of adrenaline release. Increased platelet aggregation was maintained thereafter for the two-hour test period. There was no apparent correlation with changes in cortisol, growth hormone, and prolactin. No change in platelet function was observed after the administration of L-dopa.

We suggest that the measurement of platelet aggregation during a standard insulin stress test may provide a means of evaluating platelet function in vivo and the influence of drugs thereon.

Platelets generate adenosine triphosphate as a source of metabolic energy using glucose derived from the plasma as substrate (Gross and Schneider, 1971), and thus alterations in blood glucose may modify platelet function. Increased platelet aggregability has been demonstrated in diabetic patients (Heath et al., 1971; Kwaan et al., 1972; Colwell et al., 1976), even in those allegedly well controlled with insulin. Such hyperaggregable platelets may be a more ready source of arterial smooth muscle cell stimulating factor released during aggregation (Ross et al., 1974) and hence could influence the incidence of vascular complications in diabetes. Conversely, in chronic hypoglycaemia (such as that associated with glycogen storage disease, type 1), platelet aggregation (PAG) is reduced (Hutton et al., 1976), leading to a mild bleeding disorder in some cases. A decrease in platelet adenine nucleotides (AN) has been observed in such patients, which might explain the defective PAG, since both the platelet defects and the haemorrhagic manifestations were corrected by maintaining the blood glucose at a normal level.

The present study was undertaken in the light of the above to determine whether platelet responses and their nucleotide content alter during an acute change in the blood glucose. For this purpose we investigated platelet responses and hormone changes during the transient hypoglycaemia induced by a standard insulin stress test.

Methods

Our criterion for inclusion in this series was that patients should achieve a glucose level below 2-4 mmol/l with simultaneous clinical manifestations of hypoglycaemia.

None of the patients had ingested drugs influencing platelet function in the 10 days before testing.

In each case the insulin stress test was performed for evaluation of a possible pituitary-hypothalamic disturbance, but in no instance was clinical or other evidence of this subsequently found. An indwelling venous catheter was inserted into fasting patients lying recumbent at rest, and after a 20-30 minu
'recovery' period the basal sample was collected and soluble insulin (0.1 U/kg body weight) was injected intravenously. Thereafter samples were collected at 30-minute intervals.

The catheter was connected to a three-way tap and kept patent with saline. At each sampling, 30 minutes after insulin, 5 ml (TRH), 0.2 mg, and luteinising hormone/follicle stimulating hormone releasing hormone (LH/FSH RH), 0.1 mg, were also injected and an extra blood sample was collected at 20 minutes. In three of these patients, similar studies were performed after an oral dose (250-500 mg) of levodopa (l-dopa) given to test growth hormone release.

A fraction of each blood sample was anti-coagulated with 1/10th volume of 3.8% tri-sodium citrate for the platelet studies. Platelet-rich plasma (PRP) and platelet poor plasma were prepared (Hardisty et al., 1970). The PRP platelet count was not standardised before testing since in only two cases did it vary significantly during the course of the test. Aggregation was measured by nephelometry (Born, 1962), results being expressed as percentage fall in optical density of PRP 3 minutes after the addition of aggregating agent. Several concentrations of adenosine diphosphate (ADP) were tested against the pre-insulin PRP, and that which gave an intermediate degree of aggregation (20-60% of maximum) was used for all subsequent samples. This concentration varied between 0.75 and 1.65 mM. A few patients were also tested with adrenaline, collagen, or ristocetin.

Platelet AN were measured by a modification (Hardisty et al., 1970) of the firefly luminescence method (Holmsen et al., 1966). Von Willebrand factor (ristocetin co-factor) assays were performed by the method of Weiss et al. (1973). Collagen adrenaline and ADP were purchased from Sigma Laboratories and ristocetin from Abbott Laboratories, and diluted for use in Owren's buffered saline, pH 7.35.

Results

**Symptoms during insulin stress test**

Classical symptoms of hypoglycaemia and adrenaline release (Ginsburg and Paton, 1956) were first noted about 20 minutes after insulin injection, reached a peak between 30 and 45 minutes, and had largely disappeared by 60 minutes.

**Blood glucose (Fig. 1a)**
The blood glucose fell precipitously after insulin injection, lowest levels being recorded in the 30-minute post-insulin samples. Thereafter levels rose progressively, approaching baseline values at 2 hours.

**Platelet counts**

Two of the 10 patients showed a marked, sustained rise in the platelet count of the PRP, starting 30 minutes after insulin. In a third, there was a rise at 30 minutes which had returned to around baseline by 60 minutes. In the remaining seven patients, the count did not change significantly during the test.

**Platelet aggregation (Fig. 1b)**

In the 30-minute samples, PAG (expressed as a percentage of the pre-insulin level) increased by a mean of 65% (range 12-114%), and this coincided with the onset of clinical signs of adrenaline release. The enhanced aggregation reached a plateau or, more often, fell slightly in the 60-minute post-insulin samples but subsequently rose again somewhat to a mean of 58% (29-112%) above baseline at 2 hours. Although there was a wide variation in the degree of enhancement of aggregation, the mean increase was significantly above baseline at all time intervals (p = 0.05). The injection of TRH and LH/FSH RH appeared to have no significant effect on the PAG pattern.

More variable results were obtained using other aggregating agents. Two patients tested with collagen showed increased PAG in all post-insulin samples. On the other hand, only one of four patients tested with ristocetin and none of three tested with adrenaline showed any significant increase in PAG. There were no significant fluctuations in von Willebrand factor levels in any of five patients tested.

**Platelet adenine nucleotides (Fig. 1c)**

No significant change in either the mean, total platelet AN, or mean ATP:ADP ratio was recorded. In one case, the AN level at 90 and 120 minutes post-insulin fell to 40% and 50% respectively of the pre-insulin values. The reason for this fall was not clear. A normal ATP:ADP ratio was maintained throughout, and so it is unlikely to have been the result of depletion of the storage pool AN by, for example, inadvertent generation of a trace of thrombin during blood collection.

**Hormone levels**

All 10 patients showed a normal cortisol level response, the peak occurring at 60-90 minutes. Six patients showed a rise in growth hormone which was most pronounced during the middle hour of the test, while the other four showed no change. Serum prolactin was measured in the four patients receiving TRH. All showed an increase with a peak value at 20 minutes.
STUDIES AFTER L-DOPA ADMINISTRATION
There were no significant changes in platelet count, PAG, or AN in three patients who received L-dopa. An increase in growth hormone was noted in the second hour but no adrenergic symptoms and no other hormonal change.

Discussion
The significant finding in the present study, in contrast to previous observations in chronic hypoglycaemia (Hutton et al., 1976), was that insulin-induced hypoglycaemia is accompanied by a significant enhancement of platelet aggregation, which coincides with the lowest blood glucose levels recorded and persists for at least 2 hours despite the return of blood glucose to normal.

The procedure itself did not influence the results since four patients not reported here because they achieved inadequate hypoglycaemia did not show enhanced aggregation. Two of these patients were later retested and showed increased aggregation accompanying the now adequate hypoglycaemia. Furthermore, the patients tested with L-dopa did not become hypoglycaemic and did not show enhanced aggregation.

Insulin stimulates glucose metabolism in human platelets (Karpatkin, 1967). Furthermore, platelets suspended in a glucose-free medium have decreased aggregability which is largely reversible on the addition of glucose (Kinlough-Rathbone et al., 1972), a phenomenon which may involve the prostaglandin thromboxane A2 (Horrobin and Manku, 1978). These findings show a relationship between insulin, glucose, and aggregation.

In healthy but not in adrenalectomised subjects, hypoglycaemia stimulated release of adrenaline (Ginsburg and Paton, 1956), a potent platelet-aggregating agent which acts synergistically with ADP (Ardie et al., 1966). The enhanced aggregation observed 30 minutes after insulin may thus be related to adrenaline release. In support of this explanation, platelet aggregation was almost invariably less at 60 minutes than at 30 minutes, and this decrease coincided with the decline in the clinical severity of the adrenergic response.

Also ADP enhanced aggregation while adrenaline did not in three patients tested with both agents. This could result from saturation of the adrenaline receptors.

Maintenance of enhanced aggregation during the second hour of the test cannot be attributed directly...
to continuing adrenaline release since the latter is of short duration, and all clinical signs had by then disappeared. However, it is possible that adrenaline somehow exerts a prolonged effect on platelets.

The role of other hormones should also be considered. There was no correlation between either basal cortisol levels or the extent of their rise during hypoglycaemia and platelet aggregation. However, we cannot exclude the possibility that this rise (or some associated factor) influenced platelet response.

There is ample evidence that growth hormone influences haemostasis (Merimee et al., 1970; Chiang et al., 1975; Sarji et al., 1975). However, we observed enhanced aggregation irrespective of whether or not there was a rise in growth hormone during the insulin stress test, and von Willebrand factor levels were unchanged. Moreover, there was no increase in aggregation in two patients after L-dopa when growth hormone rose in the absence of any change in blood glucose or cortisol.

Hyperprolactinaemia is occasionally a feature of certain situations associated with an increased incidence of thrombus, such as anaesthesia and ingestion of contraceptive pills, but we are unaware of any direct evidence that prolactin influences platelet aggregation, and our findings provide no indication that it does.

 Vasopressin is released during insulin-induced hypoglycaemia (Baylis and Heath, 1977) but does not cause aggregation in citrated PRP and is unlikely to have influenced our results. Similarly, there is no evidence that glucagon, which also rises during hypoglycaemia, modified platelet aggregation.

 Although at present we feel unable to account for the aggregation changes observed, the situation might be clarified by performing the test after adrenalectomy, or by blocking the cortisol and prolactin responses with cyproheptadine (Corenblum and Whitaker, 1977).

 From the practical point of view the insulin stress test provides a means of stimulating platelet function in vivo and may thus be a more appropriate model than conventional in vitro methods for evaluating antiplatelet drugs and for investigating the pathogenesis of vascular disease.

References


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