Brief Notes and Comments

Inhibition of Labile Aggregation-stimulating Substance (LASS) and Platelet Aggregation in Diabetes Mellitus

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SUMMARY

Irreversible second-phase aggregation of platelets in diabetic patients is prevented in vitro by 5, 8, 11, 14-eicosatetraynoic acid (TYA), a competitive inhibitor of the labile aggregation-stimulating substance (LASS) which is formed from arachidonic acid. Thus, inhibition of prostaglandin synthesis inhibits platelet aggregation in diabetic subjects. These findings indicate that platelets from diabetics are subject to control by intracellular mechanisms operative in the regulation of platelet function in normal individuals. DIABETES *24*:684-87, July, 1975.

Recent work by Willis has demonstrated a new factor of importance in platelet aggregation.[1] Labile aggregation-stimulating substance (LASS) is generated from arachidonic acid during the biosynthesis of prostaglandins E_2 and $F_{2\alpha}$. The production of LASS is inhibited by acetylsalicylic acid and by an acetylenic analogue of arachidonic acid, 5, 8, 11, 14-eicosatetraynoic acid (TYA).[2]

Increased sensitivity to platelet-aggregating agents has been described in diabetic patients of variable severity.[3-6] A plasma factor that interacts with platelets to produce irreversible platelet aggregation in the presence of ADP has also been described.[5] The increased platelet aggregation seen in diabetic subjects is reversed with acetylsalicylic acid therapy,[7] an inhibitor of LASS generation.[1]

In view of the fact that TYA is a more specific inhibitor of LASS generation than is acetylsalicylic acid, we have studied its effect on platelet aggregation in diabetic subjects.

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MATERIALS AND METHODS

Platelet aggregation in citrated venous blood, obtained after an overnight fast, was determined by the method of Born,[8] using a Model 300 Chronolog Aggregometer. Ten nonobese adult subjects, nine of whom had known diabetes mellitus of less than three years' duration, were studied. Seven were managed on insulin therapy and three on diet alone. Three had clinical evidence of diabetic vascular disease. All were under excellent metabolic control, with nearly normal fasting plasma levels of glucose (118 ± 13 mg./100 ml., S.E.M.), triglycerides (107 ± 11 mg./100 ml., S.E.M.), and cholesterol (199 ± 14 mg./100 ml., S.E.M.). Subjects had taken no drugs except insulin for at least two weeks.

Aggregation of platelets was induced by the addition of adenosine 5′-diphosphate, epinephrine, or collagen to platelet-rich plasma (PRP). Blood was drawn from an antecubital vein after an overnight fast and diluted 9:1 with 3.8 per cent sodium citrate. PRP was obtained by centrifuging for twenty minutes at 50 g at room temperature. All glassware was siliconized.

PPP was obtained by centrifuging the remaining sample for ten minutes at 3,300 g at 4° C. The platelet count in PPP was always less than $10{,}000/mm^3$. Platelet counts in PRP were $480{,}000 \pm 50{,}000/mm^3$ (S.E.M.).

Adenosine 5′-diphosphate (ADP) disodium salt (Sigma Chemical Co.) was dissolved in a sodium barbital buffer, pH 7.35, to give a 2-mM solution of ADP. This solution was divided into 1-ml. aliquots and frozen. One aliquot was thawed and diluted to the desired concentration with buffer for use. Concentrations shown are final plasma levels in the cuvette used for aggregation studied. Dessicated L-epinephrine (Calbiochem) was dissolved in 0.1N HCL to give a 10-mM solution. This solution was prepared daily and

diluted with buffer to the desired concentration. Collagen was prepared according to the method of Holmsen et al.[9] The free acid of TYA, in final concentrations of 150 and 300 μg./ml., was prepared in platelet-poor plasma (PPP) according to the method of Willis et al.,[2,13] and 0.05 ml. of TYA in PPP was added to 0.40 ml. of platelet-rich plasma immediately before the addition of ADP, epinephrine, or collagen.

The rates and extent of first and second phases of platelet aggregation were estimated as shown in figure 1 for ADP. Similar methods of calculation were used for aggregation after epinephrine and collagen.

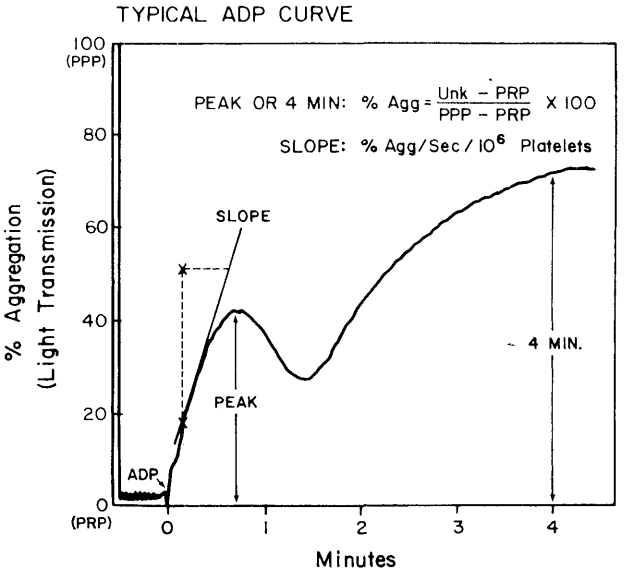

FIG. 1. Methods used for calculations of slope, peak, and four-minute values for platelet aggregation after the addition of ADP.

RESULTS

Irreversible second-phase platelet aggregation after the addition of low concentrations of ADP, epinephrine, and collagen is shown in figure 2 in one diabetic subject. TYA in a final concentration of 150 μg./ml. partially inhibited the irreversible second-phase platelet aggregation and was more active at 300 μg./ml. concentrations. The inhibition was seen with all three aggregating agents tested.

The major effect of TYA in the ten subjects studied was inhibition of the second phase of platelet aggregation after all aggregating agents tested (figure 3). Suppression was significant ($P<0.001$ by paired t-test) at both TYA concentrations in all cases. It occurred at all platelet counts in PRP. TYA (300 μg./ml.) decreased the slope of first-phase platelet aggregation after ADP (45±3 vs. 34±3 per cent, S.E.M., $P<0.01$). No other effects of the agent on any phases of platelet aggregation were found.

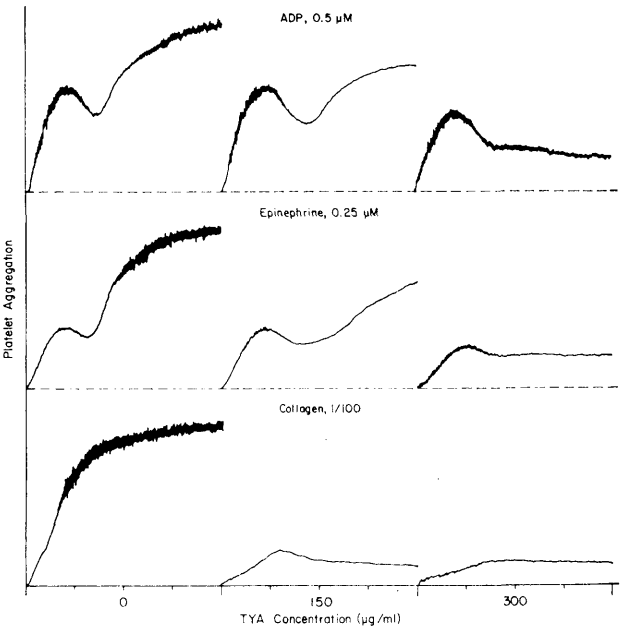

FIG. 2. Platelet aggregation after ADP, epinephrine, and collagen at concentrations of TYA of 0, 150, and 300 μg./ml.

Plasma factor activity was assayed according to a method previously described.[5] A minor potentiation of ADP-induced platelet aggregation was found when plasma from nineteen normal subjects was added to normal PRP (4.4±1.8 per cent, S.E.M.). Plasma from the ten diabetic patients gave a potentiation of 29.8±4.7 per cent. This potentiation of ADP-induced platelet aggregation by plasma factor(s) was reversed by TYA in a concentration of 300 μg./ml. (3.6±3.1 per cent, $P<0.001$).

DISCUSSION

These data support the findings of Willis et al.[2] that LASS is an important mediator of platelet aggregation. They suggest that the arachidonic acid-LASS-prostaglandin system is operative in diabetic platelets and may relate to the abnormal sensitivity of platelets from diabetic subjects to aggregating agents. The presence of previously described plasma factor activity that potentiates ADP-induced platelet aggregation is confirmed. This activity is also inhibited by TYA.

According to Willis,[1] the LASS-prostaglandin system is involved only in second-phase platelet aggregation in normal subjects. We have shown a significant

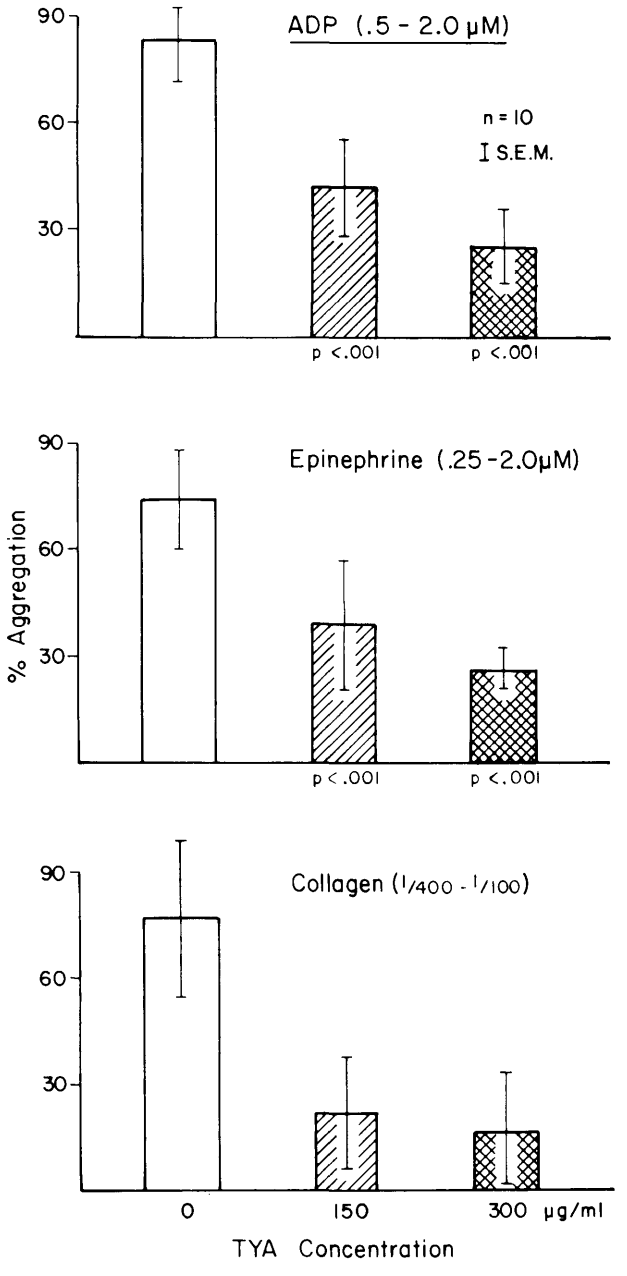

FIG. 3. Per cent of platelet aggregation four minutes after the addition of ADP, epinephrine, or collagen with varying concentrations of TYA. The shaded bars indicate results obtained with two different TYA concentrations (150 and 300 μg./ml.)

inhibitory effect of TYA on first-phase platelet aggregation after ADP, suggesting that TYA or the LASS-prostaglandin system has an influence on the initial rate of ADP-induced platelet aggregation in diabetic subjects. Although platelet damage by the higher concentration of TYA cannot be excluded, Willis found no evidence of an effect of TYA on first-phase platelet aggregation in normal subjects.[1,2]

In view of the known elevation of fatty acids in insulin-deficient diabetic subjects,[10] one can speculate that the increased platelet sensitivity to aggregating agents may be due to increased platelet or plasma arachidonic acid levels. Studies to date, however, show that fatty acids with 22:4 carbon chains make up a small fraction of the fatty acid pool of platelet phospholipids.[11,12] Increased amounts of lipid phosphorus have been found in platelets from juvenile and adult-onset diabetic subjects.[12] All major platelet phospholipid fractions were found to be elevated in the latter group. No significant elevation of the 22:4-carbon-component fatty acids of platelet phospholipids was observed. Studies of arachidonic acid uptake by platelets, its release from platelet lipoproteins, and generation of LASS and prostaglandins from arachidonic acid are now indicated in diabetic subjects. Further, the relationship between plasma factor(s) and arachidonic acid must be explored.

Finally, the in vivo significance of this system in man remains to be established. It is of interest that irreversible platelet aggregation has recently been reported in mice on intravenous LASS administration.[13]

ACKNOWLEDGMENT

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