A nucleotide with characteristic platelet aggregation and inhibition properties similar to 5-hydroxytryptamine

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The mechanism of the transient platelet aggregation response (PAR) of human platelets to 5-hydroxytryptamine (5-HT) is not well understood. We have found that adenyl-imidodiphosphate (AIP), a structural analogue of adenosine triphosphate (ATP), causes a reversible aggregation of human platelets, similar to that induced by 5-HT (Baumgartner & Born, 1968). A comparison has been made between AIP and 5-HT as inducers and as possible inhibitors of aggregation.

The extent of aggregation by AIP at a given concentration (50–100 μM) is related to the degree of PAR to 5-HT (10 μM) in the same platelet preparations. The higher the 5-HT response the greater the PAR to AIP. Another property shared by 5-HT is the fact that the transient PAR to AIP can be enhanced by short (40–60 s) preincubation with low (non-aggregating) concentrations (0.1–0.5 μM) of noradrenaline and adrenaline (Ball, Boullin & Glenton, 1977). Like 5-HT PAR, the transient PAR to AIP (50–100 μM) can be induced only once, and the platelets will not reaggregate again on further addition of the inducer. However, platelets that have aggregated reversibly to AIP, will respond by exhibiting reversible aggregation to the addition of 5-HT (10 μM). Similarity to 5-HT PAR was further strengthened by the observation that tetrahydro-β-carbolines and phenothiazines which strongly inhibit PAR to 5-HT were also potent inhibitors of PAR to AIP (Youdim, Oppenheim & Goldstein, 1978; Oppenheim, Youdim, Goldstein & Hefetz, 1978). AIP (50–100 μM), greatly enhances the first phase of adrenaline and nor-adrenaline responses, a time dependent phenomenon that is shared by 5-HT (Baumgartner & Born, 1968) and partly by ATP. AIP competitively inhibited both phases of PAR to adenosine diphosphate (ADP), however, it will inhibit only the second phase of PAR to adrenaline (10 μM) and nor-adrenaline (10 μM).

The second phase of aggregation response to ADP or adrenaline is usually related to release I, i.e. release of dense vesicle contents (Holmsen, 1975). Thus we may assume that AIP, in addition to being an aggregating agent can also be an inhibitor of release of 5-HT from storage vesicles. This possibility is presently being tested. AIP differs from ATP, adenosine monophosphate and adenosine because none of the latter compounds cause PAR at similar concentrations (50–100 μM). Since it is unlikely that a nucleotide derivative, such as AIP, will be taken up by intact platelets, one can envisage that the carrier for 5-HT uptake and its receptor for aggregation are different (Born & Michal, 1975). The lack of structural similarity between AIP and 5-HT suggests that the mechanism of reversible PAR to 5-HT may involve a nucleotide as an intermediate.

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The study of platelet aggregation in whole blood

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The invention by Born in 1962 of a turbidometric technique for measuring platelet aggregation proved to be of the greatest importance because it provided biological scientists with a tool for studying not only the mechanism of platelet aggregation *per se*, but also a host of other cellular activities which can be conveniently observed using platelets as a model system.

The main limitation of the Born aggregometer is that it only functions with translucent cell suspensions such as platelet rich plasma (PRP); in particular, it will not work in whole blood. This could be important in view of our recent finding (Blackwell, Flower, Russell-Smith, Salmon, Thorogood & Vane, 1978) that

leucocytes can generate the potent anti-aggregating agent prostacyclin and that platelets are, therefore, likely to behave differently in blood than in PRP. Another related problem is that the preparation of PRP from blood may take 15–30 min and that labile modulators of aggregation such as thromboxane A_2 (Hamberg, Svensson & Samuelsson, 1975) and prostacyclin (Moncada, Gryglewski, Bunting & Vane, 1976) may decay substantially during sample preparation. Recently, some attempts have been made to circumvent this difficulty: Gryglewski, Korbut, Ocetkiewicz & Stachura (1978) have described a bioassay technique for measuring platelet aggregation in an extracorporeal circulation and we now describe experiments designed to evaluate a novel electronic aggregometer which accepts blood as well as PRP. This is described in our demonstration (Cardinal & Flower, 1979).

In an initial series of experiments we measured the aggregation responses of citrated (or heparinised) human and rabbit PRP by the optical and electronic aggregometer in parallel. Both techniques gave dose related responses to collagen (0.1–10 μg/ml), ADP (1–20 μM), arachidonic acid (1–10 μg/ml), thrombin (0.1–1.0 U/ml) and prostaglandin endoperoxides (0.05–1.0 μg/ml) and these were antagonized by prostacyclin (1–5 ng/ml) and (with collagen, ADP and arachidonic acid) indomethacin (1–10 μg/ml). Although very similar, there were differences in the results obtained with the two techniques. The electronic aggregometer gave no 'shape-change' information, but was more sensitive, especially to collagen. Biphasic and reversible responses of human blood to ADP (1–4 μM) could be seen with the electronic aggregometer as well as the optical machine although they were not so well marked in the former. In a second series of experiments citrated (or heparinised) rabbit and human blood was used and the ability of the above agents to induce aggregation was checked. All these stimuli (same concentrations) gave similar dose-related responses to those seen in PRP and these could be antagonized by prostacyclin and (in appropriate cases) indomethacin. The electronic aggregometer gives accurate and precise measurements of platelet aggregation in blood. Quantitative studies of platelets in their natural milieu, a procedure which has never before been possible, should lead to fresh insight into the mechanisms which control clot or thrombus formation.

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A prostacyclin-like substance in rat brain

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Prostaglandin I_2 (PGI_2, prostacyclin) has been shown to have vasodilator and potent platelet antiaggregation activity (Dusting, Moncada & Vane, 1977). PGI_2 is a major product of arachidonic acid metabolism in many tissues but has not thus far been identified in brain. Sun, Chapman & McGuire (1977) studied prostaglandin and thromboxane formation in rat brain homogenates. Following incubation with the prostaglandin endoperoxide [1-^{14}C]-PGH_2 the major product was PGD_2 with lesser amounts of TxB_2 and PGE_2 and $PGF_{2\alpha}$. No PGI_2 was detected as measured by the appearance of [1-^{14}C]-6-keto $F_{1\alpha}$ in the homogenate. This method of detection is relatively insensitive; therefore, PGI_2 occurrence in rat brain has been reassessed using a sensitive biological method of detection. Wistar rats (250 g) were killed by cervical dislocation and the cerebral cortex removed. This was finely chopped and 200–500 mg placed in 1.0 ml Tris buffer pH 7.5 (0.05M) at 22°C. At various times the homogenate was rapidly centrifuged and the supernatant(S) treated in a variety of ways. Platelet aggregation activity of S was measured using an aggregometer and human PRP. 0.1 ml S or standards were added to PRP, alone or 1 min before aggression was induced with ADP (1-2 μM). 2.1 nM PGI_2 and 30nM PGD_2 could be detected.

0.1 ml S from homogenates tested immediately after 1–2 min incubation induced platelet aggregation; this is suggestive of the presence of a thromboxane-like substance which has previously been detected in rat brain (Wolfe, Rostorowski & Marion, 1976; Sun *et al.*, 1977). This activity was not further investigated.