RELEASE OF AN AGGREGATING SUBSTANCE BY HUMAN PLATELETS IN RESPONSE TO PHORBOL-ESTER STIMULATION

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The tumour-promoter, tetradecanoylphorbolacetate (Zucker et al 1974) and several 12-deoxyphorbol-esters (Westwick et al 1980) induce irreversible aggregation of human blood platelets at nanomolar concentrations. This aggregation is not primarily mediated by PG-endoperoxides, thromboxane-A_2 or ADP (Williamson et al 1981). 12-DPPA (12-deoxyphorbolphenylacetate) has an immediate and direct effect upon platelets, inducing aggregation in a structurally specific manner (ED_{50} 0.6 μm). This effect is followed by an indirect action involving a significant release reaction from the dense granules of platelets together with the release of a transferable aggregating substance (TAS). As part of the investigations into 12-DPPA induced platelet aggregation we have studied the properties of TAS.

Human blood platelet rich plasma (PRP), platelet poor plasma (PPP) preparation and the measurement of aggregation induced by 12-DPPA were carried out as previously described (Williamson et al 1981). TAS release from platelets was investigated by removing 100 μl of donor platelet suspension after 12-DPPA induced aggregation (0.86 μm) and adding it to 400 μl of fresh recipient PRP. The doses of 12-DPPA transferred in these experiments were too low to cause the resultant platelet aggregation. TAS appeared in the platelet suspension between 30 and 60 seconds after the 12-DPPA interaction with platelets and was maximal within 4 mins. but was still present 10 mins. later (Table 1).

TABLE 1

RATE OF APPEARANCE OF TAS AFTER 12DPPA INITIATED PLATELET AGGREGATION

TIME after initiation of aggⁿ by 12-DPPA (0.86 μM)	HEIGHT OF AGGᴺ CAUSED BY TAS (PRP-PPP 100mm)	
	1 min after addⁿ	4 min after addⁿ
30 sec	6mm	7mm
1 min	8mm	31mm
1 min 30 sec	21mm	36mm
2 min	33mm	54mm
2 min 30 sec	45mm	64mm
3 min	53mm	68mm
4 min	55mm	72mm
6 min	47mm	57mm
7 min	37mm	56mm
10 min	33mm	54mm

Whilst aggregation was a prerequisite for TAS production, doses of 12-DPPA which induced minimal platelet aggregation still initiated its release. TAS was not platelet bound but was found in the plasma and proved to be stable at 37°C with no appreciable degradation occurring within 30 mins, some of the aggregating activity was still present after 1 hr. Both the production and aggregation caused by TAS was inhibited by phospholipase-A_2 inhibitors, membrane stabilisers and selective inhibitors of calmodulin/Ca^{++} binding. TAS production was also affected by CP/CPK (an ADP degrading enzyme) and indomethacin but not by phenidone which inhibits both cyclo-oxygenase and lipoxygenase enzymes. The aggregation of platelets induced by TAS was blocked by an inhibitor of Ca^{++} membrane movement, CP/CPK and partly by indomethacin and phenidone.

The precise mechanism of 12-DPPA induced aggregation is unknown but available evidence indicates that 12-DPPA has a structurally specific primary effect upon platelet membranes, possibly at a receptor site, followed by a secondary indirect action involving the release of TAS together with dense granule contents of platelets.

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