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Pharmacology of the inhibition of platelet aggregation

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Many different inhibitors of platelet aggregation are known, and the pharmacology of inhibition can be divided according to the different types of inhibitors (Table 1). Aggregation of platelets is brought about directly by ADP and indirectly by other agents including thrombin, collagen particles, and the biologically active amines adrenaline, noradrenaline, and 5-hydroxytryptamine (5-HT). The direct action of ADP is antagonised specifically by ATP [4, 5]. There are specific antagonists to each of the indirectly acting agents which are the same as those antagonising their effects on other biological systems. Thrombin-induced platelet aggregation is inhibited by heparin and hirudin, which prevent the clotting of fibrinogen; aggregation by 5-HT is inhibited by methysergide and imipramine, and aggregation by adrenaline by the adrenergic α-blockers phentolamine and di-hydroergotamine [21]. The actions of agents which initiate the platelet release reaction, and the action of the ADP which is released, are also affected by a group of drugs which interfere with the release process but which do not inhibit the primary aggregation induced directly by ADP. This group includes tricyclic antidepressive drugs such as desmethyl imipramine and amitriptyline [21] and also some nonsteroidal anti-inflammatory drugs including aspirin and phenylbutazone [11]. How these release inhibitors work is unknown. We have suggested [21] that imipramine and similar compounds act by virtue of their ability to stabilize biological membranes against mechanical, chemical or osmotic damage, and so interfere with the penetration of the release stimulus into the platelet. It has recently been found that release inhibitors diminish the breakdown of about 20% of the metabolically active ATP in platelets (i. e. ATP readily labelled with radioactive precursors) which occurs when platelets are exposed to a releasing agent such as collagen [3].

The inhibitors of primary aggregation by ADP can be divided further. Aggregation depends on the presence of calcium ions, fibrinogen, ADP, and platelets in an active state [6]. Some inhibitors remove or compete with calcium and ADP; these may act also on the platelets themselves. Calcium ions are removed from solution by binding agents such as EDTA or citrate. With citrate at the concentrations commonly used for studying platelet

Table 1
Inhibitors of platelet aggregation

I. Specific antagonists of releasing agents:

1. Thrombin antagonists: heparin, hirudin
2. Serotonin antagonists: methysergide, imipramine
3. Adrenaline antagonists (α): dihydroergotamine, phentolamine*
4. Albumin – binds fatty acids

II. General inhibitors of the release reaction:

1. Membrane stabilisers: amitriptyline, desmethylimipramine
2. Anti-inflammatory agents: aspirin, phenylbutazone
3. Inhibitors of aggregation (see below)

III. Inhibitors of primary aggregation induced by ADP:

1. Calcium chelators: citrate, EDTA, ATP, excess ADP
2. Calcium competitors: TAMe, Arcaine, (H^+)
3. Structural analogues of ADP: ATP, adenosine, 2Cl-adenosine, AMP, 2-methylthio-AMP, etc.**
4. ADP destroying systems (enzymes): myokinase, apyrase, ADPase, phosphoenolpyruvate + pyruvate kinase
5. Metabolic inhibitors: iodoacetate + KCN, 2-deoxy-D-glucose + antimycin
6. Thiol reagents: p-chloromercuribenzoate, N-ethyl maleimide
7. Vasodilators: adenosine and some derivatives, prostaglandin E_1, dipyridamole, theophylline
8. Adenyl cyclase activators: prostaglandin E_1, isopropyl noradrenaline, (F^-)
9. Inhibitors of cyclic 3'5'AMP phosphodiesterase: theophylline, caffeine

* α-antagonists phenoxybenzamine and dibenamine are not active.
** Inactive compounds include IMP, inosine, adenosine 5'-sulphate.

aggregation (18–22 mM in plasma), the rate of aggregation is less than the rate when heparin is used as anti-coagulant, and this decrease varies with the species. In rats the effect of citrate is greater than in man. ATP and excessive concentrations of ADP inhibit aggregation when the calcium concentration is low [24, 29], and this effect may be due to calcium chelation. Competition with calcium ions may explain the effects of compounds such as arcaine and TAMe which contain the guanidino group [14]; the inhibition of aggregation which occurs at pH below 6.4 [18] may be due to competition between calcium and hydrogen ions for particular sites.

Agents which act directly on ADP include the enzyme which alter or inactivate the ADP molecule [12], e.g. adenylate kinase, apyrase, snake venom ADPase, and combination of pyruvate kinase with phosphoenolpyruvate as well as ADP degrading enzyme(s) present in plasma [20]. Competition between ADP and compounds related to it has been established only for ADP. Weak inhibitory activity in preparations of AMP has been attributed to contamination with or breakdown to adenosine [26]. It has been suggested that adenosine, which is rapidly taken up into platelets and incorporated into platelet nucleotides, inhibits either as a result of an in-

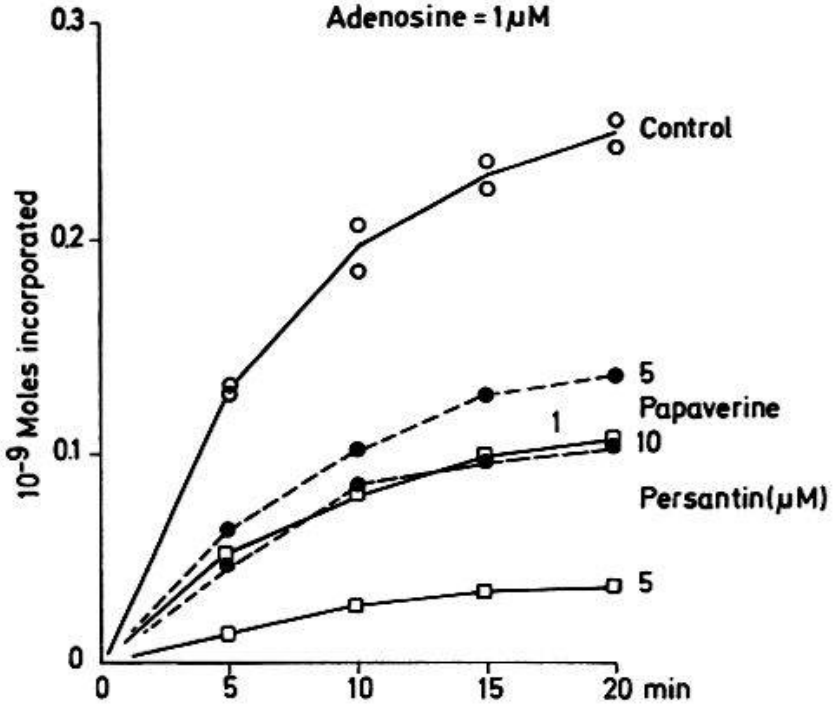

Fig. 1. Incorporation of radioactivity by platelets incubated with radioactive adenosine.

crease in platelet ATP [27], or of a localised decrease in a fraction of platelet ATP which is utilised during the initial phosphorylation of adenosine by adenosine kinase [26]. However, this is unlikely because, if it were so, drugs such as papaverine and dipyridamole (Persantin) which inhibit adenosine uptake by platelets [16] should also prevent its inhibitory effect. In fact, at concentrations of these drugs which almost completely block uptake, inhibition is actually increased [8].

Fig. 1 shows the effects of papaverine (5 and 10 μM) and dipyridamole (1 and 5 μM) on the incorporation of radioactivity by platelets incubated with radioactive adenosine. Persantin was about ten times more active than papaverine as an inhibitor of uptake. The results in Table 2 show that concentrations of papaverine which caused partial inhibition of adenosine uptake caused a considerable increase in the degree of inhibition of aggregation by adenosine. Dipyridamole in concentrations which caused almost complete inhibition of uptake also increased the inhibitory effect of adenosine on aggregation.

It seems therefore that adenosine inhibits via action on the outside of the platelets. This is consistent with the inhibitory activities of some analogues in which the 2 position of the adenine ring is substituted with the methyl-thio group. With sheep and dog platelets, 2-methyl-thio adenosine is considerably less active as an inhibitor than is 2-methyl-thio AMP, which indicates that the nucleotide must act directly rather than by being first dephosphorylated to the nucleoside. With human platelets the two compounds have about the same activity [19]. A large number of nucleosides has been tested as inhibitors of aggregation. All modifications of the adenosine mole-

Table 2

The effects of dipyridamole and papaverine on the uptake of radioactive adenosine by human platelets and on the inhibition by adenosine of platelet aggregation*

	Drug					
	Papaverine			Dipyridamole		
Concentration (μM)	1	3	10	10	30	100
% Inhibition of adenosine uptake	28	47	81	96	96	97
% Inhibition of aggregation						
Drug alone	5	3	10	2	2	4
Adenosine alone	42	39	40	33	38	36
Drug + adenosine	49	66	84	47	45	54

* Human citrated platelet-rich plasma containing 4.82×10^8 platelets/ml was incubated for 10 min with radioactive adenosine (1 μM) at 37 °C. Uptake in the absence of inhibitors was 58 pmoles/10 min/10^8 platelets. – Aggregation with ADP (2 μM) was studied in the same sample of platelet-rich plasma. Adenosine (1 μM) and the other drugs were added 2 min before ADP.

cule are less effective except those substituted in the 2 position of the purine ring [7]. There is, moreover, wide variation between species, for adenosine inhibits aggregation of platelets of man, rabbit, dog, and sheep, but not of rat, mouse, guinea-pig, hamster, and cat. These species differences are still unexplained.

The action of metabolic inhibitors, e.g. combinations of antimycin with 2-deoxy-D-glucose and of cyanide with iodoacetate, can be explained by the depletion of metabolically active ATP which appears to be necessary for aggregation. Inhibition by colchicine, a poison with a selective effect on microtubular systems, and by thiol reagents such as p-chloromercuribenzoate and N-ethyl maleimide which inactivate contractile proteins, suggests that these structures are involved in aggregation but in ways which are not understood.

Much evidence indicates that the cyclic 3'5'-adenosine monophosphate (cAMP) of platelets is involved in the control of their aggregation. High concentrations of the methyl xanthines theophylline and caffeine inhibit aggregation by ADP [2]. Platelet phosphodiesterase is inhibited by theophylline [1]. Adenyl cyclase in platelets is stimulated by adenosine and by prostaglandin E_1 (PGE_1) [13, 17, 31, 32] which inhibit aggregation.

An investigation into the nature of the platelet receptor mechanism for adrenaline [22] showed that isoprenaline inhibits aggregation by ADP. This effect is clearer when thrombin or collagen are used as aggregating agents instead of ADP. Fig. 2 shows that the inhibitory effect of isoprenaline can be prevented by the selective β-receptor blocking agent propranolol. This supported the idea of an inhibitory mechanism in platelets triggered by adrenergic β-agonists.

PGE_1 is a potent inhibitor of aggregation by ADP [15] and by other agents

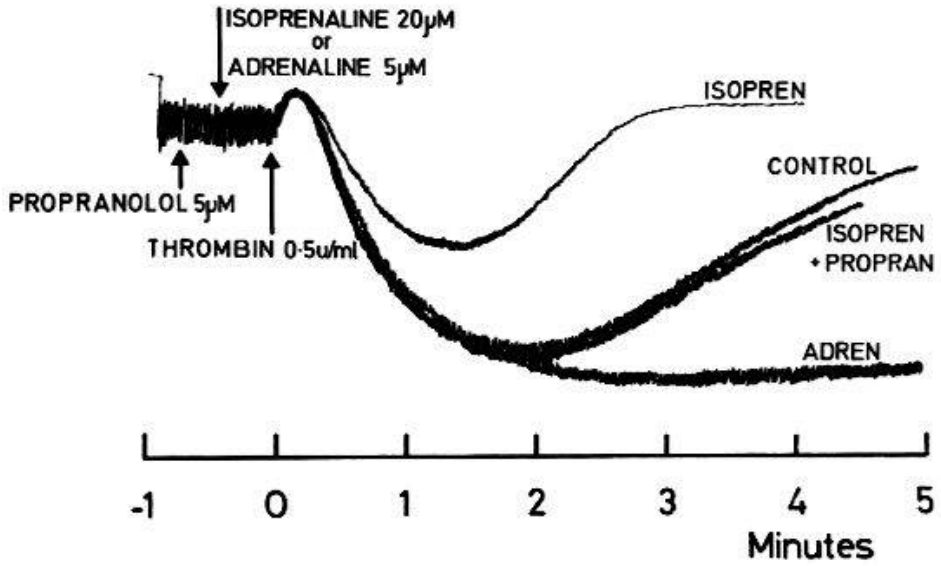

Fig. 2. Effect of propranolol and isoprenaline on platelet aggregation.

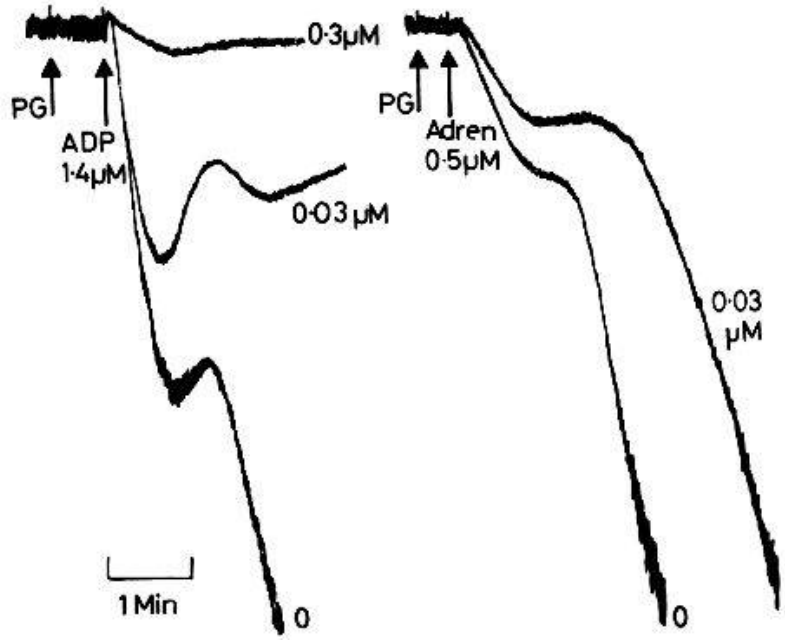

Fig. 3. Prostaglandine E_1. Inhibition of aggregation caused by ADP and adrenaline.

[10]. Its effects on aggregation are demonstrated in Fig. 3, which shows partial inhibition with a molar ratio of PGE_1 to ADP of 1:46 and of PGE_1 to adrenaline of 1:6; ADP-induced aggregation was completely blocked at a ratio PGE_1 to ADP of 1:4.6. PGE_1 is known to decrease the cAMP concentration in some tissues and to increase in others [9]; these effects are apparently due to regulation of the activity of adenyl cyclase.

If cAMP is indeed involved in controlling the responsiveness of platelets, then inhibitors of phosphodiesterase should augment the inhibitory effect of adenyl cyclase activators. Fig. 4 shows that concentrations of theophylline which by themselves have no effect on aggregation greatly increase the activity of both isoprenaline and PGE_1. On some occasions complete in-

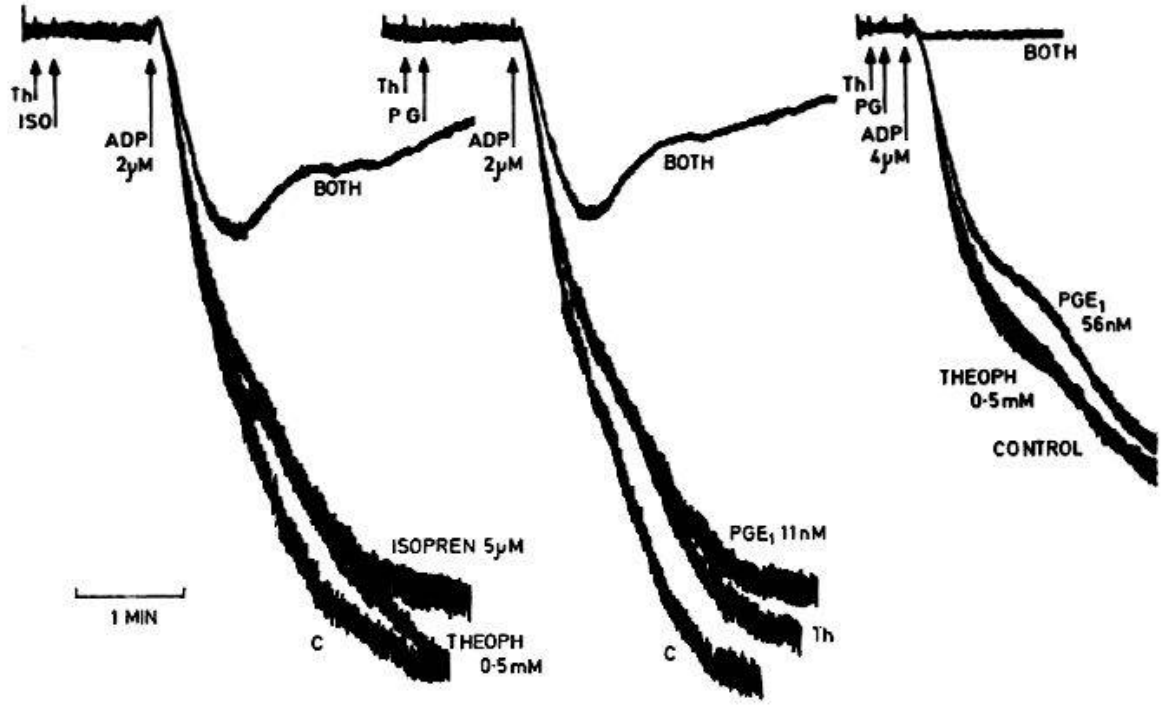

Fig. 4. Effect of theophylline on the activity of isoprenaline and PGE_1.

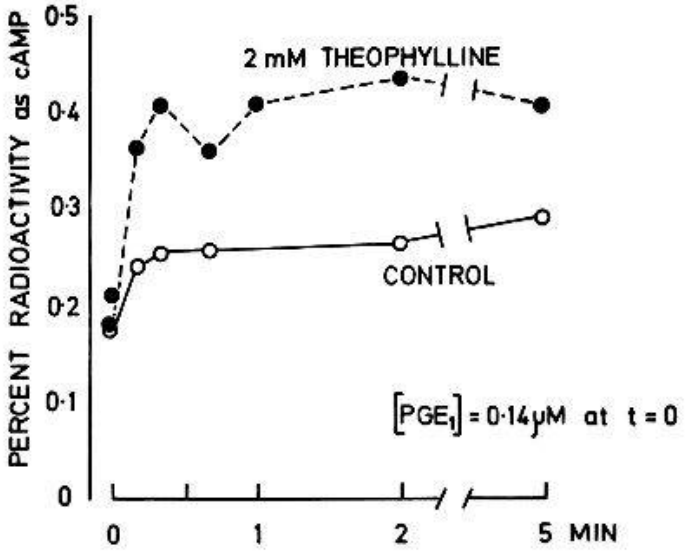

Fig. 5. Effect of prostaglandine E_1 on endogenous cyclic AMP formation in resuspended rabbit platelets.

hibition of aggregation occurred with a combination of PGE_1 and theophylline at concentrations which alone had little or no effect. In contrast to the species differences with inhibition by adenosine and its analogues, the mutual potentiation of theophylline and PGE_1 occurs in all the species so far examined including humans, rabbits, guinea-pigs, rats and hamsters.

Mutual potentiation of inhibition by theophylline and PGE_1 also occurs in suspension of platelets in tris-buffered isotonic NaCl, which aggregate on addition of ADP in the presence of calcium ions. This preparation has been useful for studying the effects of drugs on nucleotide metabolism in platelets

with nucleotides labelled with ^{14}C [3]. The results in Fig. 5 show a slight increase in counts recovered as cAMP in the absence of theophylline and a considerably greater increase when theophylline was also present. Theophylline by itself at this concentration did not cause any increase.

In order to calculate the amounts of cAMP formed in these experiments, it has been assumed that all the radioactivity was incorporated into a single pool of metabolically active nucleotides equivalent to 50% of the total platelet nucleotides, that is, that 100% of the recovered radioactivity corresponds to 6 μmoles/10^{11} platelets [23]. This gives a level of 12 nmoles/10^{11} platelets for the basal concentration and an increase to 25 nmoles/10^{11} platelets in the presence of theophylline and PGE_1. Further analysis of the chromatographically isolated cAMP by electrophoresis shows that the basal level is over-estimated by this procedure and that 6 nmoles/10^{11} platelets is probably closer to the true value.

Thus, there is now good evidence that increased intracellular cAMP is involved in the action of inhibitors such as PGE_1, isoprenaline, and methylxanthines. It is conceivable that differences in the phosphodiesterase of platelets from that in other tissues could provide a basis for a therapeutic agent with a selective inhibitory action of platelet function.

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