PLATELET AGGREGATION

IV. PLATELET PHOSPHODIESTERASE AND ITS INHIBITION BY VASODILATORS

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Platelet cyclic AMP phosphodiesterase (PDE) has been partially purified, its properties studied and inhibition by certain vasodilators observed. Quazodine, dipyridamole, papaverine, Paveril® and other agents inhibit platelet adenosine uptake, potentiate both the inhibition of aggregation and PGE_1 stimulated cyclic AMP synthesis and inhibit PDE. The mechanism of action of vasodilators as inhibitors of aggregation is discussed.

Our initial interest in prostaglandin E_1 (PGE_1), a potent vasodilator, led to the discovery that stimulation of cyclic AMP synthesis by PGE_1 occurs concomitantly with the inhibition of platelet aggregation[1] and that cyclic AMP *per se* inhibits aggregation. Therefore, we have postulated[1,2] that an increase in intracellular cyclic AMP will prevent aggregation and that regulation of cyclic AMP in the platelet could play a major role in the ultimate control of thrombogenesis.

Cyclic AMP levels can be regulated in at least two ways: by stimulation of adenyl cyclase resulting in increased synthesis of the nucleotide from ATP or by inhibition of a specific phosphodiesterase (PDE) resulting in decreased hydrolysis to 5'AMP. Papaverine has been reported to block platelet PDE[3] and methyl xanthines are well known inhibitors of PDE from other tissues[4]. However, dipyridamole, a potent coronary vasodilator and potential anti-thrombogenic agent, has been reported to function by preventing adenosine uptake[5,6]. To clarify the role of these and other vasodilators which inhibit

platelet aggregation[7,8], we have investigated the possibility that their effects are mediated through inhibition of PDE, thus increasing platelet cyclic AMP.

Materials and Methods

Adenosine-8-^{14}C was obtained from Calbiochem and ^{3}H-adenosine-3'-5' cyclic AMP from Schwarz/Mann. Whole human blood collected in 4% citrate was obtained from a local blood bank and processed as previously reported[2]. Isolated platelets from several units of blood were homogenized in 0.32M sucrose and the 100,000 x g supernatant was frozen in small volumes to be utilized as needed for PDE assay.

Platelet PDE activity was determined by measuring the rate of conversion of ^{3}H-cyclic AMP to ^{3}H-5'AMP. The incubation mixture consisted of the 100,000 x g supernatant (0.1 mg protein), ^{3}H-cyclic AMP (10 μc/μmole) from 5 x 10^{-4}M to 5 x 10^{-3}M, $MgCl_2$ (4.6 mM), tris-HCl buffer at pH 8.0 (38.5 mM) in a final volume of 0.1 ml. Routine assays were run at 37°C for 15 minutes and terminated by boiling for three minutes. Aliquots of the incubation mixture were chromatographed with a standard nucleotide mixture (ATP, ADP, AMP, cyclic AMP and adenosine) on Whatman #1 paper. The chromatographs were developed overnight using a solvent mixture of 95% ethanol and 1M ammonium acetate containing 3mM EDTA (70:30 v/v). After drying the chromatographs and delineating the nucleotides under UV light, the appropriate spots were cut out, eluted with distilled H_2O in scintillation vials, scintillation solution was added, and the vials were counted. Duplicate aliquots of incubation mixture were added to paper and counted without chromatographic development in order to determine recovery.

Platelet adenosine uptake was determined using the fol-

lowing procedure: platelets, resuspended in Tris-HCl buffered physiological salt solution (pH 7.4, 0.05M) and concentrated 20 x from PRP, were pre-incubated with or without drug in a final volume of 0.5 ml for ten minutes. Adenosine-8-^{14}C (2×10^{-4}M) was then added to each vessel and the incubation continued for another ten minutes at 37° and the tubes immediately centrifuged at 10,000 x g for five minutes. Platelet pellets were resuspended in distilled water, homogenized, and aliquots added to vials containing scintillation fluid and counted.

Results and Discussion

We have isolated and partially purified phosphodiesterase from human platelet homogenates. Ammonium sulfate fractionation of the 100,000 x g supernatant fraction of such homogenates yields a preparation between 35% and 65% of saturation which contains the bulk of platelet PDE activity. The enzyme behaves much as those isolated from other tissues; activity increases linearly with the concentration of protein and time of incubation. PDE activity increases with temperatures up to 45° and thereafter heat inactivation occurs. Full activity is observed only in the presence of added Mg++ and an optimal pH for PDE of 8.0 was determined. When routinely assayed at pH 8.0, a Km of 2×10^{-3}M is found; a lower Km of 0.3×10^{-3}M is found at pH 7.5 which is similar to that obtained for rat brain PDE[9] at this pH.

Whereas PGE_1 increases cyclic AMP in the platelet by stimulating its synthesis, the methyl xanthines increase cyclic AMP by blocking PDE. Caffeine, theophylline and aminophylline inhibit platelet PDE in a competitive fashion (Figure 1b), and the inhibition by caffeine is concentration dependent (Figure

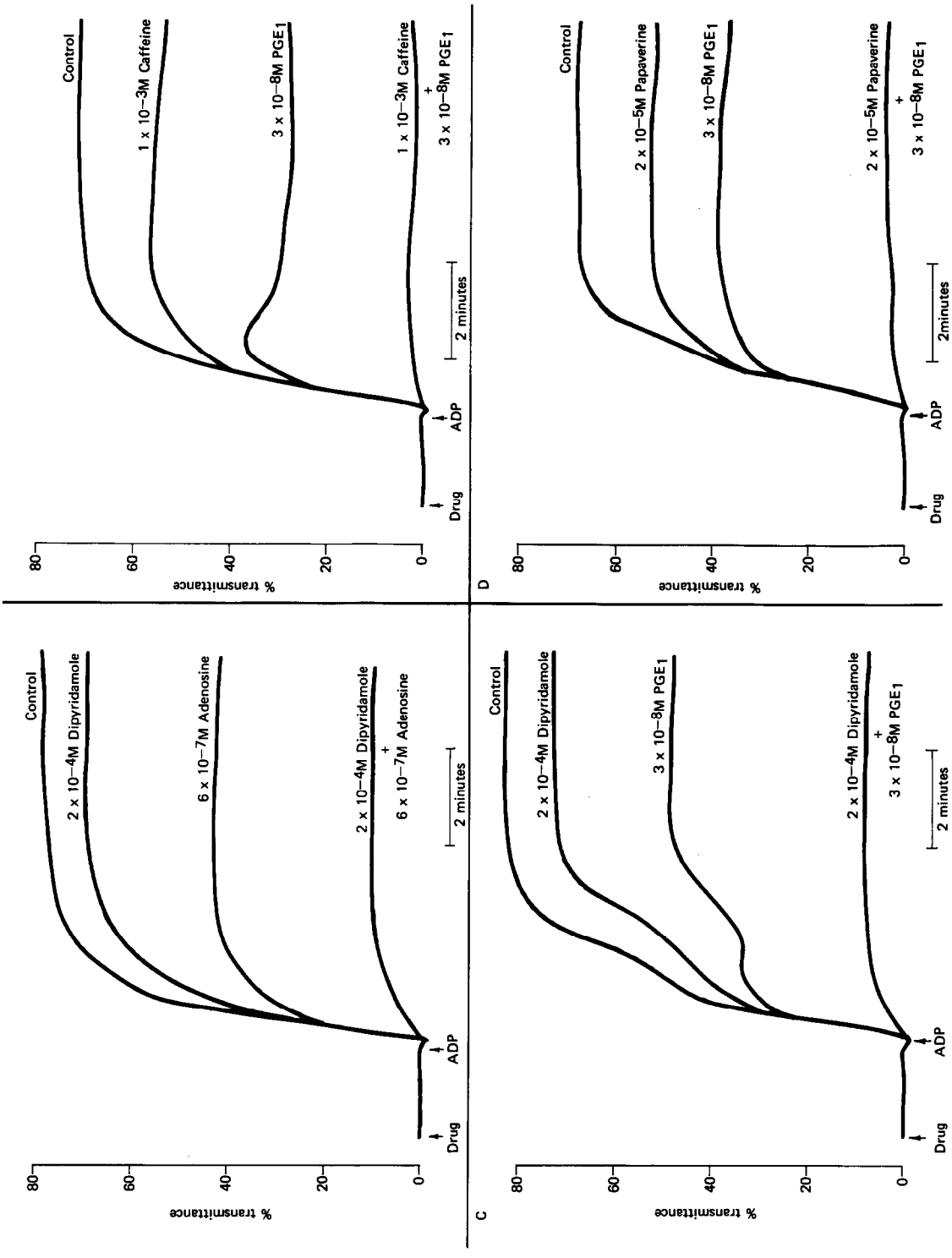

Figure 1. Competitive inhibition of platelet PDE by methyl xanthines.

1a). Platelet PDE is also inhibited by high concentrations (>1 x 10^{-3}M) of citrate and pyrophosphate as similarly reported by Cheung in the assay of rat brain PDE[9].

Certain vasodilators (papaverine, dipyridamole) have been reported to prevent adenosine uptake and/or metabolism as a possible mechanism of action in the inhibition of platelet aggregation[5,6]. However, these drugs have intrinsic inhibitory properties of their own against aggregation (Figure 2). Dipyridamole for example not only potentiates the effects of adenosine and PGE_1, but inhibits aggregation in the absence of these agents (Figure 2a,b). Papaverine too, potentiates the effects of PGE_1 (Figure 2c) but also exerts inhibition of its own. Thus, we think both of these agents must be working other

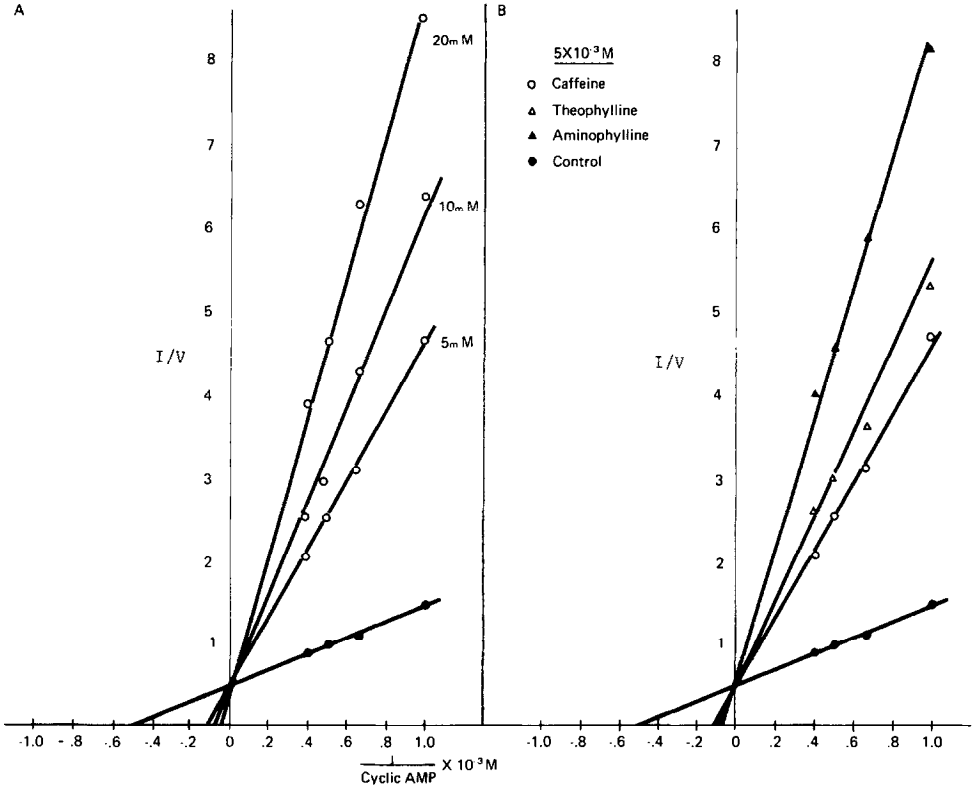

Figure 2. Potentiation of inhibition of platelet aggregation by vasodilators.

than by blocking adenosine uptake.

While several of the vasodilators affecting platelet function inhibit adenosine uptake (Table 1), neither caffeine, theophylline, PGE_1, nor cyclic AMP itself is active. Indeed, caffeine consistently stimulated uptake at high concentrations. It would appear, therefore, that elevation of cyclic AMP is not responsible for inhibition of adenosine uptake and that certain vasodilators, although blocking adenosine uptake in the platelet, do not exert their inhibition of aggregation in this manner.

As an alternative mechanism of vasodilator action on platelets, we suggest that certain vasodilators may act, like

Table 1

INHIBITORS OF PLATELET CYCLIC AMP PHOSPHODIESTERASE AND PLATELET ADENOSINE UPTAKE

Drug	Inhibitor Constant (Ki)	% Inhibition of Adenosine Uptake	
		10^{-3}M	10^{-4}M
Papaverine	4.6×10^{-6}M	73	47
Quazodine	5.0×10^{-5}M	51	45
Paveril®	5.9×10^{-5}M	59	76
Dipyridamole	1.7×10^{-5}M	68	73
Ethaverine	1.5×10^{-5}M	64	76
Theophylline	8.0×10^{-4}M	-35	-5
Caffeine	7.1×10^{-3}M	-45	-3
Lidoflazine	6.7×10^{-3}M	49	47
Verapamil	3.3×10^{-3}M	64	33
Nylindrin	10^{-2}M	12	2
Isoetharine	10^{-2}M	2	1
Nitroglycerin	10^{-2}M	-5	5

Table 2

POTENTIATION OF PGE_1 STIMULATED CYCLIC AMP SYNTHESIS BY VASODILATORS

Conditions	Nanomoles Cyclic AMP/mg Protein
PGE_1 (1×10^{-7}M)	6.5
PGE_1 + Caffeine (10^{-3}M)	12.5
PGE_1 + Papaverine (10^{-3}M)	15.4
PGE_1 + Dipyridamole (10^{-3}M)	16.5
PGE_1 + Quazodine (10^{-3}M)	20.5

PGE_1 and methyl xanthines, by increasing intracellular cyclic AMP. Inhibitory constants, calculated from kinetic data, indicate papaverine, Paveril®, dipyridamole and ethaverine to be potent inhibitors of platelet PDE (Table 1). Quazodine (6,7-dimethoxy-4-ethylquinazoline), a bronchodilator/vasodilator, is an effective inhibitor of PDE from beef brain and a number of rabbit tissues[10]. We also find quazodine to be a potent inhibitor of platelet PDE. Relatively high levels of theophylline, caffeine, lidoflazine and verapamil are necessary for half maximal inhibition. In addition, quazodine, dipyridamole and papaverine, like caffeine, potentiate PGE_1 stimulated cyclic AMP synthesis by platelet membrane fractions (Table 2). These observations, along with reports of PDE inhibition in coronary vessels[11,12] and platelets[3] by papaverine and in rat heart by intensain[13], support the possibility that certain vasodilators affect platelet aggregation by elevating cyclic AMP.

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