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Effects of papaverine derivatives on cyclic AMP phosphodiesterase of human platelets

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AGGREGATION of human platelets is inhibited by adenosine-3′,5′-monophosphate (cyclic AMP) and by its dibutyryl derivative.[1] These results suggest that cyclic AMP may be important in the regulation of platelet adhesiveness. Accordingly, the levels of intracellular cyclic AMP may play a major role in the susecptibility of platelets to aggregation and, ultimately, in thrombogenesis. The level of cyclic AMP is determined both by the activity of adenyl cyclase catalyzing the conversion of ATP to cyclic AMP, and by the activity of a specific phosphodiesterase (PDE) hydrolyzing cyclic AMP to adenosine-5′-monophosphate. Certain agents known to affect platelet aggregation are known to alter adenyl cyclase or phosphodiesterase activity in platelets. It was shown that stimulation of β-receptors by isoprenaline causes platelet clumps to disaggregate and that such a response is mediated by increased formation of cyclic AMP.[2] Prostaglandin E_1 inhibits platelet aggregation and stimulates cyclic AMP synthesis by human platelet membrane fractions apparently by stimulation of adenyl cyclase.[3, 4] On the other hand, theophylline inhibits platelet PDE resulting in increased cyclic AMP levels.[2] This drug is synergistic with isoprenaline in the inhibition of platelet aggregation.[2] We found that papaverine and some of its derivatives impair platelet functions most important for hemostasis and thrombosis.[5, 6] Platelets treated with papaverine loose their ability to adhere and to aggregate. Furthermore, phosphodiesterase activity in other tissues was found to be inhibited by papaverine.[7] Therefore, we investigated the possibility whether the inhibition of platelet aggregation by papaverine was mediated by an inhibition of platelet PDE and the subsequent increase of cyclic AMP.

The procedure used was that described by Butcher and Sutherland.[8] It involves conversion of 3′-5′ cyclic AMP to 5′ AMP, hydrolysis of the latter with excess of 5′-nucleotidase and measuring the liberated inorganic phosphate.[9] A whole platelet lysate suspension obtained by freezing and thawing of cells previously washed in saline two times and resuspended in a mixture of 2×10^{-3} M glycylglycin buffer, pH 7·4, 1×10^{-3} M $MgSO_4$, 0·01 M NaCl, and 0·01 M KCl was used for enzyme assay.

It was found that low concentrations of papaverine inhibit the PDE activity of platelet lysates. As shown in the dose–response curve (Fig. 1), 0·044 μM of papaverine/ml produce a 50 per cent inhibition of 3′-5′ cyclic AMP hydrolysis. In other experiments the effect of papaverine derivatives described as effective inhibitors of platelet aggregation[5] was compared with theophylline, a known inhibitor of 3′-5′ cyclic AMP PDE. The results are summarized in Table 1. It has been shown that all derivatives investigated are potent PDE blockers which surpass the effectiveness of theophylline.

The results support the assumption that aggregation is inhibited by substances able to induce 3′-5′ cyclic AMP accumulation in platelets.

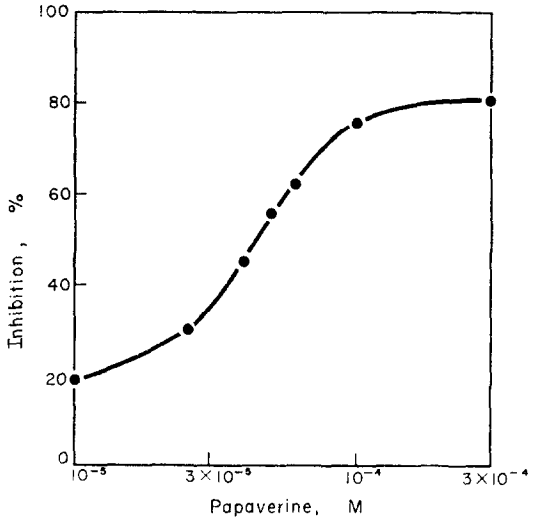

FIG. 1. Dose–response curve for the effect of papaverine on PDE activity in human platelets.

TABLE 1. EFFECTS OF PAPAVERINE DERIVATIVES ON 3′-5′ CYCLIC AMP PHOSPHODIESTERASE ACTIVITY IN LYSATES OF HUMAN PLATELETS

Addition (5×10^{-5} M)	Enzyme activity in μmoles of cyclic AMP hydrolysed per hour per milligram protein
None	0·50
Papaverine	0·22
3-Ethyl-1-benzyl-6,7-dimethoxyisoquinoline	0·26
Ethaverine	0·30
3′,4′,6,7-Tetraethoxy-1-benzyliden-1,2,3,4-tetra-hydroisoquinoline	0·36
Theophylline	0·43

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