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Inhibition of 3′5′-cyclic-AMP phosphodiesterase by some platelet aggregation inhibitors

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DURING the screening of compounds as inhibitors of 3′5′-cyclic-AMP phosphodiesterase, we chose to investigate the action of 2-chloroadenosine in view of its structural relationship to 3′5′-cyclic-AMP. 2-Chloroadenosine had previously been reported to be an inhibitor of induced platelet[1] aggregation and a potent vasodilator.[2]

We found 2-chloroadenosine to be a potent inhibitor of beef heart phosphodiesterase and from the literature we found that several known inhibitors of this enzyme have also been independently reported to be inhibitors of induced platelet aggregation. These include methyl xanthines[3, 4], phenothiazines,[5–7] intensain[8, 9] and reserpine.[5–7]

We wish to report here the action of several inhibitors of platelet aggregation on 3′5′-cyclic-AMP phosphodiesterase. The enzyme was prepared from beef heart by the method of Butcher and Sutherland[4] to the end of step 2. Incubations were carried out at 30° for 10 min in an incubation mixture containing test compound, 200 μmoles Tris-buffer pH 7·5, 2 μmoles $MgCl_2$, Crotalus adamanteus venom (0·1 mg protein) and phosphodiesterase (0·5 mg protein) in a total volume of 0·85 ml. The reaction was initiated by addition of 0·1 ml 10 mm 3′5′-cyclic-AMP and terminated with 0·05 ml 100% trichloracetic acid. Compounds which interfered with the phosphate analysis were removed by charcoal treatment. The inorganic phosphate formed from 5′-AMP by nucleotidase activity of the venom was measured by the method of Fisk and Subba-Row.[10]

Table 1 shows the 50 per cent inhibition levels obtained from graphs of at least four levels of test compounds. References describing their activity as inhibitors of platelet aggregation are also indicated.

All inhibitors were also tested for their activity on the 5′-nucleotidase of Crotalus adamanteus venom. Only 2-chloroadenosine produced any inhibition. The specific activity of the 5′-nucleotidase was much greater than that of the phosphodiesterase in the assay system used for screening and under these conditions the inhibition of 5′-nucleotidase by 2-chloroadenosine was not significant.

It is interesting to note that the rank order of potency against phosphodiesterase for 2-chloroadenosine, adenosine and adenosine N-1 oxide is the same as that observed by Born *et al.*[2] for inhibition of induced platelet aggregation. The possible significance of 3′5′-cyclic-AMP in the inhibition of

TABLE 1. INHIBITION OF 3'5'-CYCLIC-AMP PHOSPHODIESTERASE BY KNOWN INHIBITORS OF PLATELET AGGREGATION

Compound	50 per cent inhibition level in relative molarity	Platelet aggregation reference
Theophylline	2·5	3
2-Chloroadenosine	2·5	1
Adenosine	25	1
Adenosine-*N*-1-oxide	40	1
Toluidene blue	1·5	4
Promethazine	0·8	4, 5
Chlorpromazine	0·4	5

induced platelet aggregation has previously been mentioned by Ardlie *et al.*[3] in relation to the methyl xanthines. The present results make it even more necessary to establish whether platelets possess adenyl cyclase or phosphodiesterase activity so that the role of 3'5'-cyclic-AMP can be properly assessed.

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Note added in proof—The presence of adenyl cyclase in platelets has now been demonstrated by Zieve and Greenhough[11] and independently by Abdulla.[12] This latter worker has also shown that platelets possess phosphodiesterase activity.

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