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Effects of Catecholamines on the Formation of Adenosine 3′:5′-Cyclic Monophosphate in Human Blood Platelets

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Noradrenaline or adrenaline induces the aggregation of platelets in human platelet-rich plasma, whereas isoprenaline does not and can inhibit aggregation induced by ADP (Clayton & Cross, 1963; Abdulla, 1969; Mills & Smith, 1971). Studies with specific antagonists have shown that catecholamine-induced platelet aggregation is an α-adrenergic effect (Mills & Roberts, 1967), whereas the inhibitory action of isoprenaline is a β-adrenergic effect mediated by activation of platelet adenylate cyclase (Abdulla, 1969). Several studies have now shown that increased concentrations of cyclic AMP* in platelets are associated with inhibition of platelet aggregation (Salzman & Neri, 1969; Marquis, Vigdahl & Tavormina, 1969; Ball, Brereton, Fulwood, Ireland & Yates, 1970; Mills & Smith, 1971). Conversely, some aggregating agents, particularly noradrenaline and adrenaline, can inhibit platelet adenylate cyclase (Zieve & Greenough, 1969; Marquis, Becker & Vigdahl, 1970; Salzman & Levine, 1971) and decrease the elevation in platelet cyclic AMP concentration caused by prostaglandin E_1 (Robison, Arnold & Hartmann, 1969; Marquis *et al.* 1970; Moskowitz, Harwood, Reid & Krishna, 1971). There is also one report that adrenaline or ADP can decrease the basal concentration of cyclic AMP in platelets (Salzman & Neri, 1969). The observations have suggested that the aggregation of platelets by catecholamines and possibly by other agents may be mediated by a decrease in cyclic AMP. However, Haslam & Taylor (1971) found that the effects of aggregating agents added with or without prostaglandin E_1 on cyclic [^{14}C]AMP concentrations in platelet-rich plasma that had been incubated with [U-^{14}C]adenine were not consistent with this hypothesis. We have now investigated the effects of catecholamines on the formation of cyclic [^{14}C]AMP in platelets.

Methods and materials. Human blood containing 10 units of heparin/ml was centrifuged at 300*g* for 15min at room temperature to yield platelet-rich plasma containing 3.5×10^8–4.1×10^8 platelets/ml. This was incubated for 80min at 37°C with 2μM-[U-^{14}C]adenine (231–287mCi/mmol; The Radiochemical Centre, Amersham, Bucks., U.K.), by which time more than 85% of the ^{14}C was platelet-bound (i.e. removed by centrifugation). Incubations of samples of this material (0.85ml) with various additions (0.15ml; see Table 1) were terminated by mixing with 0.2ml of 3M-perchloric acid and with 0.05ml of cyclic [8-^{3}H]AMP (0.025μCi, 1.4Ci/mmol; Schwartz Bioresearch Inc., Orangeburg, N.Y., U.S.A.), which permitted determination of the recovery of cyclic [^{14}C]AMP. Precipitated proteins were removed by centrifugation at 12000*g* for 2min (Eppendorf 3200 centrifuge). The acid supernatants were passed through columns containing 1ml of AG 50W resin (X8; 200–400 mesh) (Bio-Rad Laboratories, Richmond, Calif., U.S.A.), from which cyclic AMP was eluted with water in the 2.7–6.9ml fractions. These were freeze-dried and the residues, dissolved in 0.05ml of water containing 1mM-cyclic AMP, were subjected to t.l.c. on MN-cellulose 300HR (Macherey, Nagel und Co., Düren, Germany) as described by Turtle & Kipnis (1967). This chromatography system separated ATP, ADP, AMP, IMP, inosine, adenosine, hypoxanthine, xanthine and adenine from cyclic AMP. Cyclic AMP was eluted from the cellulose with water and the ^{14}C and 3H present were assayed by liquid-scintillation counting in a dioxan-based phosphor (Scales, 1967). A Philips liquid-scintillation counter was used, giving counting efficiencies for ^{14}C and ^{3}H of about 44% and 9% respectively. Corrections for background, channel cross-over and quenching were applied by the Philips computer. The total disintegrations recorded as cyclic [^{14}C]AMP ranged from 2000 to 15000 in 10min. These values were corrected for the recoveries of cyclic [8-^{3}H]AMP (30–50%) and expressed as percentages of the platelet-bound ^{14}C.

Papaverine, (−)-noradrenaline and (−)-adrenaline were obtained from British Drug Houses Ltd., Poole, Dorset, U.K., and phentolamine was from Ciba Laboratories, Horsham, Sussex, U.K. (−)-Isoprenaline and (−)-propranolol were kindly supplied by Dr R. Howe of these laboratories. Catecholamines were used as bitartrates and other bases as hydrochlorides.

* Abbreviation: cyclic AMP, adenosine 3′:5′-cyclic monophosphate.

Table 1. *Accumulation of cyclic [^{14}C]AMP in labelled platelet-rich plasma incubated with papaverine and various catecholamines and antagonists*

Portions (0.85 ml) of radioactive heparinized platelet-rich plasma were incubated for 1 min or 2 min at 37°C with 0.15 ml of the various additions dissolved in 0.154 M-NaCl. Final concentrations are indicated. Each incubation in each experiment was duplicated and mean values for the percentage of cyclic [^{14}C]AMP present at the end of the incubations are given. Platelet counts in the incubation mixtures were: A1, 3.18×10^8/ml; A2, 3.15×10^8/ml; B1, 3.50×10^8/ml; B2, 3.00×10^8/ml. Concentrations of cyclic [^{14}C]AMP at zero time in platelet-rich plasma mixed with saline were: A1, 0.031%; A2, 0.038%; B1, 0.071%; B2, 0.058%.

	Cyclic [^{14}C]AMP (% of total platelet ^{14}C)			
Experiment no. ...	1		2	
Incubation period ...	1 min	2 min	1 min	2 min
Additions				
A saline	0.030	0.031	0.039	0.036
Papaverine (2 mM)	0.061	0.086	0.066	0.097
Papaverine (2 mM)+(−)-noradrenaline (10 μM)	0.058	0.080	0.049	0.078
Papaverine (2 mM)+(−)-adrenaline (10 μM)	0.079	0.105	0.077	0.094
Papaverine (2 mM)+(−)-isoprenaline (10 μM)	0.208	0.251	0.243	0.260
B saline	0.064	0.056	0.059	0.053
Papaverine (2 mM)	0.129	0.190	0.038	0.129
Papaverine (2 mM)+phentolamine (5 μM)	0.134	0.214	0.095	0.143
Papaverine (2 mM) +(−)-propranolol (5 μM)	0.131	0.211	0.100	0.136
Papaverine (2 mM)+phentolamine (5 μM)+(−)-propranolol (5 μM)	0.138	0.219	0.091	0.132
Papaverine (2 mM)+(−)-adrenaline (4 μM)	0.159	0.190	0.100	0.119
Papaverine (2 mM)+(−)-adrenaline (4 μM)+phentolamine (5 μM)	0.271	0.295	0.166	0.181
Papaverine (2 mM)+(−)-adrenaline (4 μM)+(−)-propranolol (5 μM)	0.086	0.129	0.070	0.068
Papaverine (2 mM)+(−)-adrenaline (4 μM)+phentolamine (5 μM)+(−)-propranolol (5 μM)	0.130	0.172	0.087	0.111

Results and discussion. Experiments were performed in the presence of 2 mM-papaverine, a potent inhibitor of platelet cyclic AMP phosphodiesterase (Markwardt & Hoffmann, 1970), to facilitate detection of changes in adenylate cyclase activity caused by the catecholamines. Addition of papaverine alone to the platelet-rich plasma caused an approximately linear accumulation of cyclic [^{14}C]AMP during the period studied, but the amounts accumulating varied by a factor of two in different platelet-rich plasmas, as did the basal concentrations observed without papaverine (Table 1). Noradrenaline (10 μM) inhibited this accumulation of cyclic [^{14}C]AMP to a variable extent, whereas in contrast adrenaline (4 μM or 10 μM) caused a reproducible increase in the cyclic [^{14}C]AMP present after 1 min incubations, corresponding to a 40–60% stimulation of platelet adenylate cyclase over this period. In 2 min incubations the overall effect of adrenaline varied because the stimulation of adenylate cyclase was often replaced during the second minute by an inhibition relative to the controls with papaverine alone (e.g. Table 1, A2, B1 and B2). This sequence of events casts doubt on the relevance to adrenaline-induced aggregation of measurements of platelet cyclic AMP made after 1 min (see, e.g., Salzman & Neri, 1969). The effects of isoprenaline (10 μM) qualitatively resembled those of adrenaline, but the increase in cyclic [^{14}C]AMP during the first minute was much more marked, corresponding to a 600–700% stimulation of adenylate cyclase. During the second minute the further increase in cyclic [^{14}C]AMP was much diminished and was sometimes less than in the control with papaverine alone (e.g. Table 1, A2).

The contributions of α- and β-adrenergic receptors to the effect of adrenaline on platelet adenylate cyclase were evaluated by using the specific antagonists phentolamine and propranolol (Table 1). These drugs alone caused slight increases of doubtful significance in the accumulation of cyclic [^{14}C]AMP caused by papaverine. In the presence of papaverine and phentolamine, adrenaline caused a large increase in the accumulation of cyclic [^{14}C]-AMP, corresponding in 1 min incubations to a 350% activation of adenylate cyclase, which is much greater than that observed without phentolamine. This effect was followed by an inhibition of the accumulation of cyclic [^{14}C]AMP during the second minute. In contrast, when phentolamine was replaced by propranolol, adrenaline powerfully inhibited the accumulation of cyclic [^{14}C]AMP

during both the first and second minutes. When both phentolamine and propranolol were present adrenaline had little effect in 1 min incubations.

These findings establish that the action of adrenaline on the formation of cyclic [^{14}C]AMP in intact platelets is the product of the opposing effects of stimulation of α- and β-receptors, and that in the absence of antagonists the balance of these effects favours increased adenylate cyclase activity in the first minute, during which adrenaline-induced aggregation, an α-adrenergic effect, begins. These findings cannot be reconciled with the general hypothesis that α-adrenergic effects are mediated by inhibition of adenylate cyclase (Robison, Butcher & Sutherland, 1970) without invoking compartmentation of platelet adrenergic receptors as well as of platelet adenylate cyclase and its associated ATP. It seems more probable that the decrease in cyclic [^{14}C]AMP formation, which occurs after stimulation of platelet α-receptors alone, is a secondary effect that does not mediate aggregation. Possible indirect mechanisms of adenylate cyclase inhibition include depletion of substrate ATP by competing reactions or exposure to inhibitory Ca^{2+} ions. The present results do not eliminate the possibility that α-adrenergic antagonists could increase platelet cyclic AMP phosphodiesterase activity in the absence of papaverine (see Amer, 1971), but against this Haslam & Taylor (1971) found that adrenaline powerfully inhibited the accumulation of cyclic [^{14}C]AMP induced by prostaglandin E_1 in both the presence and the absence of caffeine. Thus adrenaline increases the formation of platelet cyclic [^{14}C]AMP in the absence of prostaglandin E_1 but decreases it in the presence of prostaglandin E_1. This implies that the effects of β-receptor stimulation and of prostaglandin E_1 on platelet adenylate cyclase are not additive.

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