PLATELET AGGREGATION

III. An Epinephrine Induced Decrease in Cyclic AMP Synthesis

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Epinephrine decreases Prostaglandin E_1-stimulated and basal cyclic AMP synthesis of intact platelets and of platelet membrane fractions. This effect of epinephrine is in turn inhibited by the α-adrenergic antagonist, phentolamine, but not by the β-adrenergic antagonist, propranolol. The epinephrine-mediated decrease in cyclic AMP in relation to platelet function is discussed.

Mills, et al. (1), have shown that epinephrine induces aggregation and potentiates ADP-mediated aggregation of human platelets. The order of activity of the catecholamines as inducers of aggregation was shown to be: epinephrine > norepinephrine > isoproterenol, with the latter in fact being inactive or inhibitory. This classification (2) suggests that the catecholamine induction of aggregation is mediated by an α-adrenergic mechanism. Further supporting evidence is provided by the observations that α-adrenergic antagonists such as phentolamine (1,3,4,5) prevent epinephrine-induced aggregation, whereas β-adrenergic antagonists such as propranolol (5) are without effect, except at very high concentrations.

Wolfe, et al. (6), and Marquis, et al. (7), independently showed that PGE_1, a potent inhibitor of ADP and epinephrine-induced aggregation, stimulates cyclic AMP synthesis by platelet membrane fractions. Marquis, et al. (7), have in addition shown that both cyclic AMP and its dibutyryl derivative

inhibit ADP and more recently epinephrine and collagen-induced aggregation (8). Since catecholamines have been demonstrated to affect the adenyl cyclase system of various tissues (9) it has become important to determine if the mechanism of catecholamine-induction of aggregation involves cyclic AMP. Recently Zieve and Greenough (10) in platelet lysates, Salzman and Neri (11) in platelet rich plasma and Marquis, et al. (8), in isolated intact platelets and in platelet membrane fractions reported that epinephrine decreased the synthesis or the level of cyclic AMP. This communication substantiates and extends these earlier observations.

Materials and Methods

Epinephrine bitartrate, phenylephrine HCl, phentolamine methanesulfonate, and propranolol HCl were obtained commercially. All concentrations of drugs prepared are expressed in terms of the free base.

The prelabeling of intact platelet nucleotide pools by incubation with ^{14}C-adenosine and the assay of cyclic AMP synthesis in intact platelets and by platelet membrane fractions has been described (7,12).

Results and Discussion

Epinephrine significantly decreases PGE_1-stimulated cyclic AMP synthesis both in isolated intact platelets (Figure 1, A and B, Table 2) and in platelet membrane fractions (Table 1). When isolated platelets are pre-incubated with PGE_1 so as to increase the level of intracellular cyclic AMP, the addition of epinephrine results in a marked and rapid decrease in the level of cyclic AMP synthesized (Figure 1A). The inhibition of cyclic AMP synthesis by epinephrine in intact platelets is concentration dependent as shown in Figure 1B.

In preliminary experiments, we experienced difficulty in demonstrating significant decreases in basal nonstimulated cyclic AMP synthesis with epinephrine. The addition of a

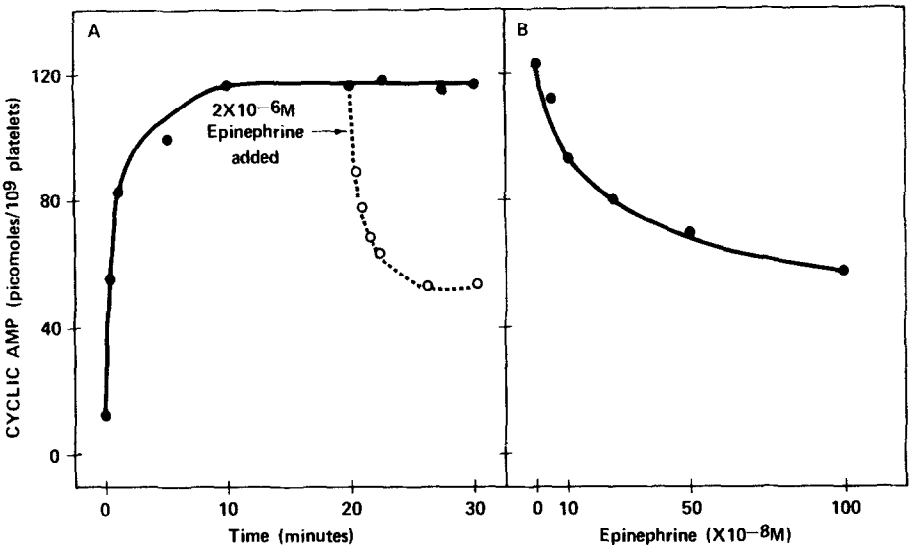

Figure 1. Human platelets isolated from platelet rich plasma were resuspended in a Tris-HCl buffered physiological salt solution containing EDTA and pre-incubated with ^{14}C-adenosine as previously described (12). 1-A and 1-B. All vessels containing ^{14}C-labeled platelets were pre-incubated with PGE_1 (10^{-7}M) for 20 minutes prior to the addition of epinephrine. 1-B, the incubation was terminated 20 minutes after adding epinephrine.

phosphodiesterase inhibitor, caffeine, was perhaps responsible. As shown in Table 1, epinephrine significantly decreases basal C-AMP synthesis by platelet membrane fractions in the absence of caffeine. The addition of caffeine, however, markedly decreases the epinephrine effect. With stimulation of cyclic AMP synthesis by PGE or NaF in the absence of caffeine, epinephrine markedly decreases the level of cyclic AMP (Table 1). Addition of caffeine in this case also results in an inhibition of the epinephrine effect.

Phentolamine, an α-adrenergic antagonist, essentially prevents the lowering of basal and PGE_1-stimulated cyclic AMP synthesis by epinephrine in platelet membrane fractions (Table 1) and in intact platelets (Table 2). Propranolol, a β-adrenergic antagonist, is without effect. Phenylephrine, a purer but weaker α-adrenergic agonist, also decreases PGE_1-

TABLE 1

CYCLIC AMP SYNTHESIS BY HUMAN PLATELET MEMBRANES

Conditions*	Cyclic AMP (nanomoles/mg protein)
Control	1.64
Epinephrine (Epi)	1.24
Caffeine (Caff)	2.19
Epi + Caff	1.92
Prostaglandin E_1 (PGE_1)	5.37
Caff + PGE_1	24.40
Epi + PGE_1	1.53
Epi + Caff + PGE_1	18.40
NaF	2.33
Epi + NaF	1.36
Phentolamine (Phent)	1.64
Epi + Phent	1.58
Phent + Caff + PGE_1	24.96
Phent + Caff + Epi + PGE_1	23.00

*Agents were present in a final concentration of: epinephrine, 2×10^{-6}M; caffeine, 2×10^{-2}M; prostaglandin E_1, 1×10^{-7}M; NaF, 1×10^{-2}M; and Phentolamine, 1×10^{-6}M.

stimulated cyclic AMP synthesis (Table 2), and its effect is also prevented by phentolamine.

Robison, et al. (13), and Turtle, et al. (14), have previously proposed that α-adrenergic stimulation might result in a decrease of cyclic AMP levels. If the decrease in cyclic AMP synthesis in platelets induced by epinephrine, blocked by phentolamine but not by propranolol is indicative of α-adrenergic mediation, then indeed a decrease in cyclic AMP level may mediate α-adrenergic responses.

Our previous observations (7,12) and those reported above

TABLE 2

ALPHA-ADRENERGIC BLOCKADE OF EPINEPHRINE OR PHENYLEPHRINE-MEDIATED DECREASE OF CYCLIC AMP SYNTHESIS IN INTACT PLATELETS

Conditions*	Cyclic AMP (picomoles/10^9 platelets)
Control	12.0
Epinephrine	9.4
Phentolamine	13.5
Propranolol	12.8
Prostaglandin E_1 (PGE_1)	57.6
Epinephrine + Phentolamine	12.9
Epinephrine + Propranolol	9.5
PGE_1 + Epinephrine	25.6
PGE_1 + Phenylephrine	40.9
PGE_1 + Epinephrine + Phentolamine	52.9
PGE_1 + Phenylephrine + Phentolamine	60.3
PGE_1 + Epinephrine + Propranolol	26.5

*Agents were present in a final concentration of: epinephrine, 2×10^{-6}M; phentolamine, 1×10^{-6}M; propranolol, 1×10^{-6}M; phenylephrine, 1×10^{-5}M; prostaglandin E_1, 1×10^{-7}M; and caffeine, 2×10^{-2}M, was present in all incubations of isolated intact platelets.

suggest that the intracellular level of cyclic AMP may regulate the aggregability of platelets. As depicted in Figure 2, agents which increase cyclic AMP by stimulation of adenyl cyclase (PGE_1) or by inhibition of phosphodiesterase (methyl xanthines) inhibit aggregation or induce disaggregation; whereas agents which induce (epinephrine) or potentiate (imidazole) aggregation, may do so by inhibiting adenyl cyclase or stimulating phosphodiesterase respectively. Alternatively, cyclic AMP levels may be altered by the depletion or accumulation of substrate ATP. It appears unlikely that epinephrine stimulates ATP depletion in our system since we have observed at high concentrations of ATP

(4×10^{-3}M), the same degree of inhibition with epinephrine as at low concentrations of substrate.

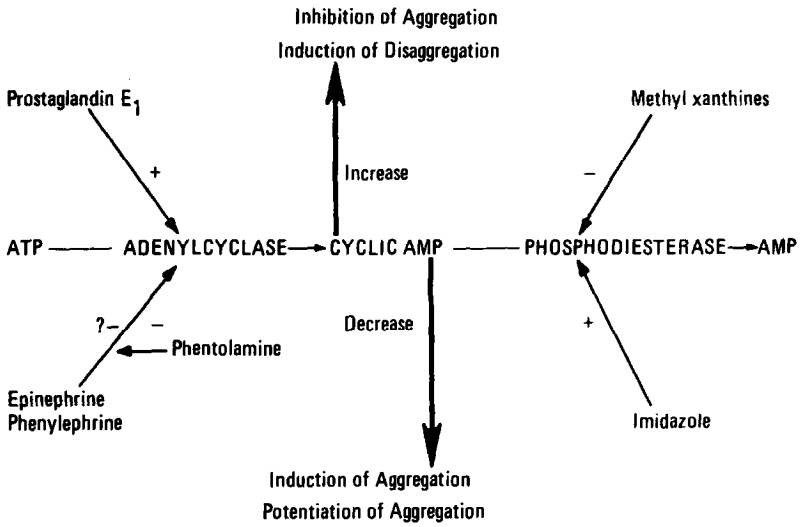

Fig. 2

Epinephrine does not affect the activity of our crude cyclic AMP phosphodiesterase preparation, and we obtain stimulation of enzyme activity only with imidazole under certain conditions (15). In contrast, Dr. M. S. Amer, in a personal communication, revealed that epinephrine stimulated partially purified cyclic AMP phosphodiesterase prepared from a number of rabbit tissues (e.g., brain, liver). Our observations that caffeine significantly prevents an epinephrine decrease in cyclic AMP in our system might favor such a mechanism. Perhaps the recent observations of Cheung (16) wherein he has reported a dissociation of a stimulating factor upon purification of phosphodiesterase may be pertinent to the above observations. The existence of such a mechanism in platelets could be of considerable importance in regulating hemostasis and thrombogenesis.

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