ADENYL CYCLASE IN HUMAN PLATELETS: ACTIVITY AND RESPONSIVENESS

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Summary

Hormonally responsive adenyl cyclase activity was measured in lysates of human platelets. Activity was stimulated by fluoride, prostaglandin E_1, and glucagon; and was inhibited by thrombin, epinephrine, norepinephrine, and serotonin. The results suggest that adenosine-3′, 5′ monophosphate (cyclic AMP) may be important in the regulation of platelet adhesiveness.

Introduction

The human platelet is a readily accessible cell in which a number of important biologic processes can be measured. Many of these processes are dependent upon the breakdown of adenosine-tri-phosphate (ATP). In other cells conversion of ATP to the cyclic nucleotide, adenosine-3′, 5′ monophosphate (cyclic AMP), (1) is one of the ways in which ATP is utilized. Cyclic AMP has been shown to be linked, in these systems, to essential cellular functions, which are often hormonally regulated (1). The level of cyclic AMP is determined both by the activity of an enzyme, adenyl cyclase, (2), which catalyzes the conversion of ATP to cyclic AMP, and by a specific phosphodiesterase which hydrolyzes cyclic AMP to adenosine-5′-monophosphate (AMP) (3). In the current experiments activity of adenyl cyclase was demonstrated in the human platelet and shown to be responsive to hormonal stimulation.

Methods

Platelets were isolated from venous blood drawn from normal donors, as previously described (4). The cells were washed twice with 2 ml of isotonic Tris buffered saline, pH 7.4, and sedimented by centrifugation at 22,000 g for 5 minutes. The tubes were swabbed with a cotton-tipped applicator to

remove free fluid, and the platelet buttons were then weighed. The pellets were resuspended in sufficient buffer to make a final concentration of 25 mg platelets (wet weight) per ml. The suspensions were rapidly frozen in a dry-ice-ethanol bath and thawed at 37°C. Final incubation mixtures consisted of 20 uL of homogenized platelets, 10 uL of 0.1 M caffeine, 10 uL of aqueous solutions of a variety of test substances, and 10 uL of a solution containing 25 uC/ml of ^{14}C ATP (410 mC/mM, New England Nuclear Corp., Boston, Mass.) and .03 M magnesium chloride. The mixtures were incubated at 37° C in an oscillating Dubnoff Shaker for 10 minutes unless otherwise specified. The reactions were stopped by placing the tubes in boiling water for 3 minutes. In all experiments a control tube was exposed to boiling for 3 minutes prior to incubation. Thereafter, 0.5 ml of a carrier solution was added to each tube. This solution contained .007 M ATP and .0025 M cyclic AMP in 0.1 M Tris buffer, pH 7.4. The mixtures were then centrifuged at 5600 g for 5 minutes. The entire supernatant from each tube was placed on 3 cm columns of Dowex 50 W - X8, 100-200 mesh, hydrogen form, packed in Pasteur pipettes. The columns were washed with 2 ml water; and the effluent, containing residual ATP, was discarded. Remaining nucleotides were eluted with an additional 3 ml of water. Nucleotides other than cyclic AMP were precipitated by the addition of 0.2 ml of balanced solutions of zinc sulfate and saturated barium hydroxide. Two ml of the supernatant solution were added to 15 ml of a scintillation medium (5) and counted in a Packard Tri-Carb Scintillation Counter. The results were expressed as disintegrations per minute (DPM). In all instances the DPM of the preboiled samples were subtracted from those of the test samples. The mean activity in the preboiled samples was 234 ± 114 DPM/mg wet platelet in 9 experiments. Specificity of the isolation of cyclic AMP was confirmed by chromatographic separation of known ^{14}C labeled nucleotides. There was no contamination with ATP or AMP in the final medium. Recovery of the cyclic AMP was 30 per cent of that added to and incubated with preboiled lysate.

Results

The mean activity of adenyl cyclase in platelet lysates was 1243 DPM/mg wet platelets in 9 experiments (range 756 - 2164, SD ± 179). In 5 experiments the mean activity of lysates incubated in the absence of caffeine did not exceed that measured in the preboiled samples. Preliminary experiments indicated that the optimal final concentration of lysed platelets was 10 mg per ml [equivalent to 2.4 mg dry weight per ml (5)] or a total of approximately 2 x 10^7 lysed platelets in the incubation mixture. The amount of cyclic AMP measured increased with the time of incubation for 8 to 10 minutes and then, in the next 50 to 52 minutes remained constant or decreased.

Incubation of lysates with sodium fluoride in a final concentration of 1 x 10^{-2} M increased measured activity of adenyl cyclase 12 fold (range 7 to 26 fold) in 7 experiments.

Prostaglandin E_1 (PGE_1) at final concentrations of 20 ng/ml and above increased by 3 to 7 fold the activity of adenyl cyclase in 7 experiments. Perceptable stimulation was observed at concentrations as low as 2 ng/ml.

Three fold stimulation of adenyl cyclase activity was also observed after incubation of lysates with glucagon, 40 mg/ml in 3 experiments.

Bovine thrombin (Parke Davis and Co.), 200 U/ml final concentration, reduced activity of adenyl cyclase 76% (7 experiments). Epinephrine, 2 ug/ml and norepinephrine, 2 ug/ml, (final concentrations) inhibited activity of adenyl cyclase 19% (7 experiments) and 26% (5 experiments) respectively. Serotonin (2 ug/ml) reduced activity 67% (5 experiments).

Discussion

These studies have demonstrated adenyl cyclase activity in the human platelet and have shown that this activity can be stimulated and inhibited *in vitro*. Measured activity was dependent on the presence in the incubation medium of caffeine, an inhibitor of the specific phosphodiesterase which

converts cyclic AMP to AMP (3). As in other systems, fluoride ions markedly stimulated cyclase activity (2).

PGE_1 is one of a number of naturally occurring prostaglandins, which are found in many human tissues as well as in circulating blood. It has been demonstrated that PGE_1 decreases the level of cyclic AMP in isolated rat fat cells and increases levels of cyclic AMP in rat lung, diaphragm, and spleen (6). The activity of adenyl cyclase in lysates of human platelets is responsive to PGE_1 at extremely low levels (2 ng/ml).

The effects of glucagon and epinephrine on adenyl cyclase in other cells have been reviewed recently (1). Both of these substances have been shown to stimulate activity of this enzyme in a variety of tissues. It has been shown that B adrenergic drugs produce an increase in levels of cyclic AMP and a commensurate stimulation of adenyl cyclase (1) whereas adrenergic drugs depress levels of cyclic AMP in these tissues. The effect of epinephrine, in the present experiments, appears to be mediated primarily through adrenergic receptors.

The functions of the human platelet which are mediated through cyclic AMP have not been determined. However, it is interesting to note that PGE_1, a potent stimulator of adenyl cyclase activity, inhibits aggregation of platelets by a variety of substances (6) and that thrombin, epinephrine, norepinephrine, and serotonin, agents known to aggregate platelets under appropriate conditions (7), inhibit adenyl cyclase activity in the platelet.

It is conceivable that an active process is required to diminish the tendency of platelets to adhere to each other or to other tissues. The breakdown of ATP to cyclic AMP may be important in this process.

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