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Characterization of α- and β-Adrenergic Receptors Linked to Human Platelet Adenylate Cyclase

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Summary. Adrenaline and noradrenaline cause aggregation of human platelets through α-adrenergic receptors, whereas isoprenaline through β-adrenergic receptors can inhibit aggregation. Either type of adrenergic receptors is coupled to platelet adenylate cyclase. Stimulation and inhibition of adenylate cyclase by β- and α-adrenergic stimulants, respectively, had been demonstrated in human platelet lysates. These effects were characterized with regard to the effectiveness of various agonists and antagonists.

Reduction of platelet adenylate cyclase activity was observed only with L-adrenaline and L-noradrenaline. This inhibitory effect, which was increased in the presence of a β-adrenergic blocking agent, was half-maximal at about 1 to 2×10^{-6} M adrenaline, and maximal inhibition (by 50–60%) was observed at about 3×10^{-5} M. Various other catecholamine and imidazoline derivatives that act as α-adrenergic agonists in other cell types neither induced aggregation nor affected the enzyme activity.

Adrenaline-induced inhibition of platelet adenylate cyclase was prevented by α-adrenergic blocking agents. These compounds inhibited the effects of adrenaline on aggregation and on adenylate cyclase with similar efficacies. Dihydrogenated ergot alkaloids were more effective than phentolamine and yohimbine; phenoxybenzamine, tolazoline and azapetine were least effective. Adrenaline-induced inhibition of platelet adenylate cyclase was reversed by phentolamine without apparent lag phase.

In the presence of α-adrenergic blocking agents, adrenaline was capable of increasing adenylate cyclase activity between 20 and 50%. Only adrenaline and isoprenaline stimulated adenylate cyclase activity; other compounds that stimulate β-adrenergic receptors in other cell types, including β_2-adrenergic stimulants, had no effect on the activity of the platelet enzyme. The stimulatory effect of adrenaline was prevented by various β-adrenergic blocking agents including pindolol and propranolol. Preferentially β_1-adrenergic receptor blocking agents such as practolol and atenolol were without effect.

These findings indicate that the spectrum of compounds capable of exhibiting intrinsic activity through α- and β-adrenergic receptors of human platelets is very narrow and that either type of platelet adrenergic receptors appears to differ from those found in other cell types.

Key words: Adenylate cyclase – Platelets – α-Adrenergic receptors – β-Adrenergic receptors – Adrenaline effects.

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Introduction

Human platelets respond to stimulation by adrenaline and noradrenaline with aggregation (O'Brien, 1963). This effect involves α-adrenergic receptors (O'Brien, 1964; Mills and Roberts, 1967). Agents that inhibit platelet aggregation such as prostaglandin E_1, prostaglandin D_2, prostacyclin and adenosine cause an increase in platelet cyclic AMP levels and stimulate cyclic AMP formation in cell-free preparations of platelets (Wolfe and Shulman, 1969; Salzman and Neri, 1969; Haslam and Lynham, 1972; Mills and Macfarlane, 1974; Gorman et al., 1977). Adrenaline and noradrenaline have been found to reduce the effect of these agents to increase platelet cyclic AMP levels and to lower cyclic AMP levels that had previously been increased. The fall in cyclic AMP levels could be blocked by α-adrenergic blocking agents but not by β-adrenergic blocking agents (Salzman and Neri, 1969; Robison et al., 1969; Marquis et al., 1970; Cole et al.,

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1971; Haslam and Taylor, 1971; Harwood et al., 1972). In contrast to adrenaline and noradrenaline, isoprenaline is a weak inhibitor of platelet aggregation. This effect involves β-adrenergic receptors and is accompanied by increased formation of cyclic AMP (Abdulla, 1969; Haslam and Taylor, 1971).

We have recently demonstrated an inhibition of basal and stimulated adenylate cyclase (EC 4.6.1.1) in lysates of human platelets by adrenaline and noradrenaline (Jakobs et al., 1976). The inhibitory effect of these catecholamines was reversed by the α-adrenergic blocking agents, phentolamine and dihydroergotamine, and was enhanced by the β-adrenergic blocking agents, propranolol and pindolol. In contrast, adrenaline stimulated platelet adenylate cyclase in the presence of the α-adrenergic blocking agents, phentolamine and dihydroergotamine (Jakobs et al., 1976). We have extended these studies in order to characterize the inhibitory and stimulatory effects involving α- and β-adrenergic receptors, respectively, on adenylate cyclase activity. This paper shows that only some of the compounds that interact as agonist or antagonist with adrenergic receptors in other cell types are capable of affecting platelet adenylate cyclase and that neither type of platelet adrenergic receptors can be classified with regard to established subclasses.

Materials and Methods

a) Reagents. ATP, creatine phosphate and creatine kinase (EC 2.7.3.2, 25 U/mg) were from Boehringer Mannheim. 3-Isobutyl-1-methylxanthine (IBMX) was purchased from Aldrich Chemical Corp. [α-^{32}P]ATP was prepared by the method of Symons (1974) as modified by Nakai and Brooker (1975) with a specific activity between 50 and 100 Ci/mmol at the time of preparation. Carrier-free [^{32}P]phosphoric acid used for this preparation was obtained from New England Nuclear Corp.

The bitartrates of L-adrenaline and L-noradrenaline, L-phenylephrine hydrochloride and yohimbine hydrochloride were purchased from Sigma. Synephrine tartrate, L-isoprenaline bitartrate, orciprenaline sulfate, bamethan sulfate, fenoterol hydrobromide and the hydrochlorides of clonidine, etilefrine and toliprolol were provided by Boehringer Ingelheim. Methoxamine hydrochloride was a gift of Wellcome, Großburgwedel; pholedrine sulfate was provided by Knoll AG, Ludwigshafen, naphazoline nitrate and the hydrochlorides of xylometazoline, phentolamine, tolazoline and oxprenolol by CIBA-Geigy, Basel, oxymetazoline hydrochloride by E. Merck, Darmstadt, and tetryzolin hydrochloride by Pfizer, Karlsruhe. The methansulfonates of dihydroergotamine, dihydroergocryptine, dihydroergocristine and dihydroergocornine and D,L-pindolol hydrochloride were provided by Sandoz, Nürnberg and Basel, azapetine phosphate by Hoffmann-La Roche, Grenzach, the hydrochlorides of atenolol, practolol, L- and D-propranolol by ICI-Pharma, Plankstadt, alprenolol hydrochloride by Regis Chemicals, Chicago, buphenine hydrochloride by Karl Thomae, Biberach, isoxsuprine hydrochloride by Troponwerke, Köln, and phenoxybenzamine hydrochloride by Röhm and Haas, Darmstadt. Aequous solutions of adrenergic agonists and antagonists were prepared shortly before experiments.

Neutral aluminum oxide for column chromatography (type 90, activity grade I) was obtained from E. Merck, Darmstadt. All other reagents were from commercial sources and of the highest purity available.

b) Preparation of Human Platelet Lysates. Platelets were prepared from blood of healthy volunteers who had not taken any medication for at least one week prior to collection, essentially as previously described (Jakobs et al., 1976). Lysates of platelets were obtained by rapid freezing of washed platelet preparations in liquid nitrogen, storage at −70° and thawing shortly before adenylate cyclase assays.

c) Adenylate Cyclase Assay. Adenylate cyclase reactions were carried out essentially as described (Jakobs et al., 1976). Activity was determined in a reaction mixture containing 50 mM triethanolamine-HCl buffer, pH 7.4, 0.1 mM [α-^{32}P]ATP (0.3–0.8 μCi per tube), 5 mM $MgCl_2$, 1 mM IBMX, 0.1 mM ethyleneglycol-bis(β-aminoethylether)*N,N'*-tetraacetic acid (EGTA), 5 mM creatine phosphate and 0.4 mg/ml creatine kinase in a total volume of 100 μl. Reactions were initiated by the addition of platelet lysate (100–200 μg of protein) to reaction mixtures that had been preincubated for 5 min at 37 °C. Incubations were carried out in duplicate (in the time course experiments) or triplicate for 10 min except when indicated. Cyclic AMP formation was linear as a function of time for at least 20 min and as a function of platelet lysate protein up to 250 μg of protein per tube.

Adenylate cyclase reactions were terminated by the addition of 0.5 ml of a solution of 0.12 M zinc acetate. Cyclic AMP was isolated by coprecipitation of related 5'-nucleotides and inorganic phosphate with $ZnCO_3$ formed by the addition of 0.6 ml of 0.12 M Na_2CO_3 and by column chromatography on neutral alumina, essentially as described (Jakobs et al., 1976). Cyclic AMP recovery through the isolation procedure was more than 98% (after correction for aliquoting) and not changed by any of the reagents used. Standard deviations of triplicate tubes were generally less than 5% of the means. Comparable results to those shown were obtained in at least two separate experiments in each case.

d) Other Methods. Protein was determined as described by Lowry et al. (1951), using bovine serum albumin as standard. Platelet aggregation was monitored in platelet-rich plasma anticoagulated by 0.38% (w/v) sodium citrate (Born, 1962).

Results

A. Effects of Adrenaline

Adrenaline reduced adenylate cyclase activity in platelet lysates in a concentration-dependent manner when added between 3×10^{-7} and 10^{-4} M (Fig. 1, left panel). The inhibitory effect of adrenaline was half-maximal at about 10^{-6} M and maximal at about 3×10^{-5} M; adrenaline at this concentration caused about 20% inhibition. Adrenergic blocking agents had divergent effects on the adrenaline-induced inhibition (Fig. 1, right panel). In the presence of the β-adrenergic blocking agent, pindolol, the inhibitory effect of adrenaline was increased; maximal inhibition was about 50%. Again, maximal and half-maximal inhibition occurred at about 3×10^{-5} M and 10^{-6} M adrenaline, respectively. In the presence of the α-adrenergic blocking agent, phentolamine, adrenaline caused a concentration-dependent stimulation of adenylate cyclase