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Agonistic and Antagonistic Effects of Various α-Adrenergic Agonists in Human Platelets

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Summary. In human platelets, the effects of various α-adrenergic agonists were studied on platelet aggregation and adenylate cyclase activity. Out of many phenylethylamine derivatives tested, only some catecholamines were able to act as α-adrenergic agonists inducing platelet aggregation and inhibition of adenylate cyclase with the order of potency: adrenaline > noradrenaline > α-methylnoradrenaline. Other phenylethylamine and imidazoline derivatives, which act as potent α-adrenergic agonists in various systems, neither induced primary aggregation nor adenylate cyclase inhibition, when tested at concentrations up to 1 mM. Since binding studies indicated high affinities of these agents to the platelet α-adrenergic receptor, their effects on adrenaline-induced aggregation and adenylate cyclase inhibition were studied.

Both types of α-adrenergic agonists tested, phenylethylamine and imidazoline derivatives, prevented adrenaline-induced aggregation and adenylate cyclase inhibition. The imidazolines, xylometazoline, oxymetazoline, naphazoline, clonidine and tetryzolin, were the most effective antagonists with similar potencies as observed with the typical α-adrenergic antagonists, phentolamine and yohimbine. Phenylethylamine derivatives such as phenylephrine, methoxamine, synephrine and norfenefrine, similarly antagonized the adrenaline-induced responses, but higher concentrations were required. The potencies of these phenylethylamine derivatives were similar to those of the classical α-adrenergic antagonists, phenoxybenzamine and azapetine. The results indicate that the platelet α-adrenergic receptor, which has many similarities with the α_2-adrenergic receptor with regard to affinities of various α-adrenergic agonists, completely differs from that found in other tissues, inasmuch as only some catecholamines acted as agonists whereas other phenylethylamine derivatives and imidazoline derivatives acted as antagonists.

Key words: Platelets – α-Adrenergic receptors – Platelet aggregation – Adenylate cyclase inhibition.

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A preliminary report of these studies has been presented (Jakobs, 1978)

Introduction

Based on the order of potency of different catecholamines, the existence of two distinct types of catecholamine receptors, α- and β-adrenergic, has been proposed (Ahlquist, 1948). β-Adrenergic receptors were later subdivided into β_1- and β_2-subtypes on the basis of organ selectivity of a spectrum of β-adrenergic agonists (Lands et al., 1967). A subclassification of α-adrenergic receptors has been recently developed from the finding that in some tissues presynaptic α-adrenergic receptors greatly differ from postsynaptic α-adrenergic receptors in their apparent affinity to agonists (Starke, 1972; Starke et al., 1974, 1975). It has been proposed that adrenergic receptors resembling presynaptic α-adrenergic receptors should be classified as α_2, and those resembling postsynaptic α-adrenergic receptors as α_1 (Langer, 1974; Berthelsen and Pettinger, 1977). According to this nomenclature and to the potency ratios orginally found for vascular pre- and postsynaptic α-adrenergic receptors (Starke et al., 1974, 1975), phenylephrine and methoxamine are prototypes of α_1-adrenergic agonists, whereas clonidine, α-methylnoradrenaline and tramazoline are prototypes of α_2-adrenergic agonists (Berthelsen and Pettinger, 1977). It should be noted that 'α_1' and 'α_2' are pharmacological receptor categories and are not synonyms of 'postsynaptic' and 'presynaptic', respectively, since for instance, receptors of the α_2 type occur at sites other than nerve terminals (see Berthelsen and Pettinger, 1977).

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In human platelets, adrenaline and noradrenaline induce primary aggregation (O'Brien, 1963, 1964; Mills and Roberts, 1967; Bygdeman and Johnson, 1969). Since this effect is blocked by α-adrenergic antagonists such as dihydroergotamine, phentolamine and phenoxybenzamine, α-adrenergic receptors are assumed to mediate this catecholamine response. We have recently shown that in lysates and particles of human platelets α-adrenergic agonists inhibit adenylate cyclase (Jakobs et al., 1976, 1978a). Comparison of the effects of various α-adrenergic agonists and antagonists on platelet aggregation and adenylate cyclase activity revealed a close correlation (Jakobs et al., 1978b). α-Adrenergic antagonists such as dihydroergotamine and phentolamine, which were potent inhibitors of adrenaline-induced aggregation, were also potent inhibitors of the adrenaline effects on adenylate cyclase activity, and vice versa, weak inhibitors of aggregation such as phenoxybenzamine and azapetine were also less potent to prevent the adrenaline effects on adenylate cyclase activity. Out of many α-adrenergic agonists tested, only adrenaline and noradrenaline induced primary platelet aggregation and inhibition of adenylate cyclase, whereas other phenylethylamine derivatives such as phenylephrine and methoxamine and all imidazoline derivatives tested neither caused platelet aggregation nor reduced adenylate cyclase activity.

Recently, α-adrenergic receptors have been identified in human platelets using [^{3}H]dihydroergocryptine (Kafka et al., 1977; Newman et al., 1978; Alexander et al., 1978) or [^{3}H]dihydroergonine (Jakobs and Rauschek, 1978) as labelled ligands. In these studies, the binding sites for the labelled α-adrenergic antagonists showed the typical pattern of an α-adrenergic receptor with regard to the binding affinities of various agonists and antagonists. In contrast to the data obtained studying platelet aggregation and adenylate cyclase activity (Jakobs et al., 1978b), a great number of agonists besides adrenaline and noradrenaline showed high affinities to this receptor. Especially imidazoline derivatives such as xylometazoline, oxymetazoline and clonidine displayed affinities that were about two orders of magnitude higher than those of adrenaline and noradrenaline, i.e., the affinities of these agents ranked in the order of the most potent α-adrenergic antagonists.

Agents that bind to the receptor with high affinity, but are unable to induce biological responses, should behave as antagonists. We tested this hypothesis, and the data presented show that out of many α-adrenergic agonists, tested on aggregation and adenylate cyclase activity, only the catecholamines, adrenaline, noradrenaline and α-methylnoradrenaline, elicited biological responses, whereas other phenylethylamine derivatives and imidazoline derivatives had no intrinsic activities and behaved as α-adrenergic antagonists in human platelets.

Materials and Methods

Materials and methods used were essentially the same as previously described (Jakobs et al., 1976, 1978b).

a) Reagents. ADP and GTP were purchased from Boehringer, Mannheim. Prazosin hydrochloride was obtained from Pfizer, Karlsruhe, norfenefrine hydrochloride from Gödecke, Berlin, and *l*-α-methylnoradrenaline hydrochloride was a gift of Dr. K. Starke, Freiburg, and of Hoechst, Frankfurt. All other reagents were obtained as previously described (Jakobs et al., 1978b).

b) Preparation of Platelets and Platelet Particles. Platelets were obtained from blood of healthy volunteers who had not taken any medication for at least 2 weeks prior to collection (Jakobs et al., 1976). For the studies of platelet aggregation in platelet-rich plasma, blood was anticoagulated by 0.38% (w/v) sodium citrate. Platelet particles were prepared by rapid freezing and thawing of the isolated platelets, centrifugation of the platelet lysates at 30,000 × g for 20 min and resuspension of the pellets in 10 mM triethanolamine-HCl buffer, pH 7.4, containing 145 mM NaCl (Jakobs et al., 1978a).

c) Adenylate Cyclase Assay. The standard reaction mixture for the determination of adenylate cyclase activity contained in 50 mM triethanolamine-HCl buffer, pH 7.4: 0.1 mM [α-^{32}P]ATP (0.3 to 0.8 μCi/tube), 5 mM $MgCl_2$, 0.1 mM ethyleneglycol-bis(β-aminoethylether)N,N'-tetraacetic acid (EGTA), 1 mM 3-isobutyl-1-methylxanthine, 5 mM creatine phosphate and 0.4 mg/ml of creatine kinase (25 U/mg); the total volume was 0.1 ml. GTP (30 μM) and the β-adrenergic blocking agent, pindolol (10 μM), were included under each condition. Reactions were initiated by the addition of platelet particles (30–60 μg of protein), conducted for 10 min at 37° C and terminated by the addition of 0.6 ml of 0.12 M zinc acetate. Cyclic AMP formed was isolated by coprecipitation of related 5'-nucleotides on $ZnCO_3$ and subsequent column chromatography on neutral alumina as previously described (Jakobs et al., 1976). Cyclic AMP formation was linear as a function of time for at least 20 min under these conditions. Standard deviations of triplicate tubes were generally less than 5% of the means.

d) Platelet Aggregation. Platelet aggregation was monitored in platelet-rich plasma at 37° C in an ELVI-aggregometer as described by Born (1962). Platelet-rich plasma (230 μl, about 250,000 platelets/μl) was added to plastic cuvettes and stirred at 1,000 rpm. After 5 min, aggregation was initiated by the addition of the catecholamines or ADP at the indicated concentrations and monitored for another 10 min. The changes of light transmission were expressed as percent in a scale in which the transmission values obtained with platelet-rich plasma and platelet-poor plasma were defined as zero and 100% transmission, respectively. For the studies of α-adrenergic antagonism, the test compound was added at the indicated concentrations 1 min prior to the addition of 1 μM adrenaline. In order to quantify and to compare the effects of various agonists and antagonists, the slope of the initial linear decrease in optical density (primary aggregation) was evaluated (cf. Fig. 3). The fast decrease in optical density follwed thereafter (secondary irreversible aggregation) is mediated by released substances (prostaglandins, ADP) at threshold concentrations, and is, once going on, not affected by α-adrenergic antagonists. Therefore, the initial slope of the primary aggregation is a better parameter than the total difference in optical density, especially at high agonist and small antagonist concentrations. For comparison of platelets from different donors