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INHIBITION OF ADRENALINE-INDUCED PLATELET AGGREGATION BY THE α-ADRENOCEPTOR BLOCKING DRUG BENEXTRAMINE

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1 The activity of the irreversible α-adrenoceptor blocking drug, benextramine, was determined in human platelets.

2 Compared to its postsynaptic and presynaptic α-adrenoceptor blocking potency, benextramine had a very low potency as an antagonist of adrenaline-induced platelet aggregation.

3 The results confirm the previous observation with the irreversible α-adrenoceptor blocking drug, phenoxybenzamine, that platelet α-adrenoceptors differ from postsynaptic and presynaptic α-adrenoceptors.

Introduction

The purpose of this study was to evaluate the activity of benextramine on platelet α-adrenoceptors. Benextramine is an irreversible α-adrenoceptor blocking drug (Melchiorre, Yong, Benfey & Belleau, 1978).

The α-adrenoceptors of human platelets are often said to be similar to presynaptic α-adrenoceptors, but there are differences. For example, the irreversible α-adrenoceptor blocking drug, phenoxybenzamine, has a high presynaptic α-adrenoceptor blocking potency but a very low potency as an antagonist of adrenaline-induced platelet aggregation.

Benextramine has a low potency as an antagonist of adrenaline-induced platelet aggregation, and relatively high concentrations had to be used. Therefore we evaluated the effect of benextramine on platelet aggregation caused by other agents. We also compared the effects of benextramine with those of the adrenaline antagonists, phentolamine, quinidine and verapamil.

Methods

Blood was collected from healthy subjects in Fisher 10 ml Vacutainer tubes (containing 143 USP units of heparin) using 20-gauge Vacutainer needles. The tubes were centrifuged at 100 *g* for 12 min and the platelet-rich supernatant was kept at room temperature.

Changes in light transmission as a result of platelet aggregation were measured at 37°C in a Dual Channel Aggregation module of Payton Associates (Scarborough, Ontario) and recorded by a Fisher Recordall 5000 recorder.

The aggregating agents were adrenaline (1 μM), ADP (1 or 2 μM), and 0.01 ml of the collagen preparation of Holmsen, Day & Storm (1969). They were added to the platelet-rich plasma to give a final volume of 0.4 ml.

Adrenaline causes immediate partial aggregation followed by a lag phase and rapid and complete aggregation. The slope of the initial (first phase) aggregation is linearly related to the log of the dose (O'Brien, 1963). Adenosine 5'-diphosphate (ADP) causes an immediate partial aggregation in small and complete aggregation in larger concentrations. Collagen causes complete aggregation after a lag phase. The extent of inhibition of aggregation was calculated for the adrenaline effect from the slope of the first phase and for the effects of ADP and collagen from the height of the response.

Incubation with the antagonists was 30 min for benextramine, 10 min for phentolamine, 5 min for quinidine, and 2 min for verapamil. Control experiments were repeatedly carried out during the course of every experiment by determining the aggregation response to the various agonists to assure the consistency of platelet aggregation.

The drugs used were: (−)-adrenaline bitartrate (Winthrop), benextramine (N,N'-*bis* (6-[*o*-methoxybenzylamino]-*n*-hexyl)cystamine tetrahydrochloride monohydrate; Aldrich), collagen (from bovine Achilles tendon; Sigma), phentolamine methane sulphonate (Ciba), quinidine sulphate

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(Merck), sodium adenosine 5'-diphosphate (Sigma), and verapamil hydrochloride (Knoll).

Results

Figure 1 shows that 10 μM benextramine slightly inhibited, 30 μM benextramine greatly inhibited and 100 μM benextramine completely inhibited the adrenaline effect. The results are summarized in Figure 4. Benextramine inhibited the effect of 1 μM adrenaline 100% in a concentration of 100 μM, 70% in a concentration of 30 μM, and 14% in a concentration of 10 μM. When these data are plotted as a log dose-response curve, the IC_{50} value for benextramine is 20 μM.

Phentolamine had a much higher potency, causing 51% inhibition of the adrenaline effect in a concentration of 100 nM. The potency of quinidine was greater than that of benextramine; it inhibited the adrenaline effect 68 and 89%, respectively, in 10 and 30 μM concentrations. The potency of verapamil was similar to that of benextramine; it caused 71% inhibition in a concentration of 30 μM.

The benextramine antagonism of ADP concentrations lower than 1 μM was not studied. Figure 2 shows that 30 μM benextramine inhibited the ADP effect slightly and 100 μM benextramine, partially. Figure 4 summarizes the results. Benextramine inhibited the ADP effect 6 and 56%, respectively, in concentrations of 30 and 100 μM. When these data are plotted as a log dose-response curve, the IC_{50} value for benextramine is 83 μM. The other drugs affected the ADP-induced aggregation only slightly.

Figure 3 shows that 30 μM benextramine partially inhibited and 100 μM benextramine completely inhibited the collagen effect. The results are summarized in Figure 4. Benextramine caused 10 and 100% inhibition of collagen-induced aggregation in concentrations of 30 and 100 μM respectively. When these data are plotted as a log dose-response curve, the IC_{50} value for benextramine is 50 μM. Both quinidine and verapamil antagonized the collagen effect (IC_{50} 63 μM and 50 μM, respectively), but the effect of phentolamine was small.

Discussion

Benextramine has a low affinity for the α-adrenoceptors in human platelets, being 200 times less potent than phentolamine (IC_{50} 20 μM and 100 nM, respectively). In contrast, 0.3 μM benextramine inhibits the noradrenaline-induced contraction of the rabbit aorta partially following 30 and 60 min incubation and completely after a 90 min incubation (Melchiorre *et al.*, 1978).

Benfey, Melchiorre & Belleau (unpublished) evaluated the presynaptic α-adrenoceptor blocking activity of benextramine. Benextramine and phentolamine increased the positive inotropic effect of high-voltage stimulation in guinea-pig isolated atrium, presumably by increasing the release of noradrenaline from sympathetic fibres. Half-maximal potentiation of the inotropic effect of high-voltage stimulation occurred with 2.3 μM benextramine and 100 nM phentolamine. The sympathomimetic drug, clonidine, decreased the effect of high-voltage stimulation, presumably by decreasing the release of noradrenaline. The clonidine effect was antagonized competitively by phentolamine and noncompetitively by benextramine. Phentolamine (1 μM) prevented the noncompetitive blockade by 3 μM benextramine and thus protected the presynaptic α-adrenoceptors against benextramine.

Thus benextramine has a higher affinity for postsynaptic and presynaptic α-adrenoceptors than for

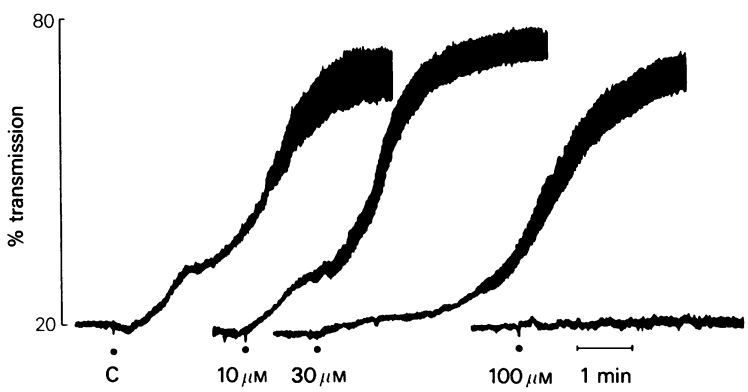

Figure 1 Platelet aggregation by 1 μM adrenaline in the absence (C) and presence of 10 μM, 30 μM, and 100 μM benextramine.

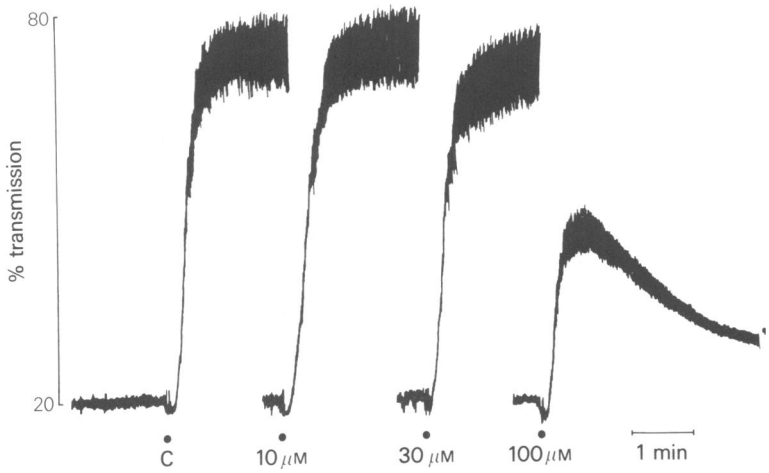

Figure 2 Platelet aggregation by 2 μM ADP in the absence (C) and presence of 10 μM, 30 μM, and 100 μM benextramine.

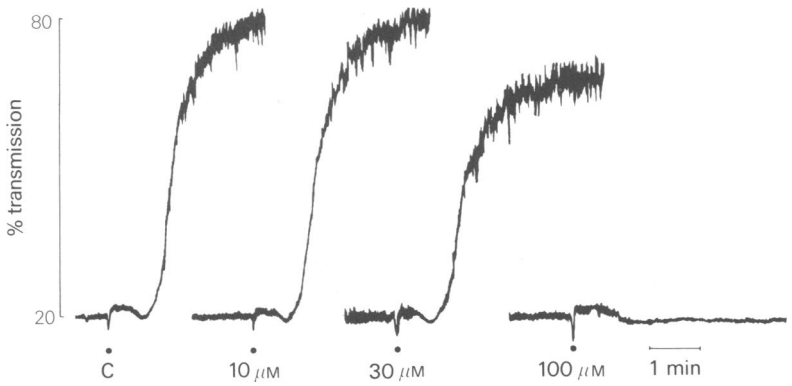

Figure 3 Platelet aggregation by collagen in the absence (C) and presence of 10 μM, 30 μM and 100 μM benextramine.

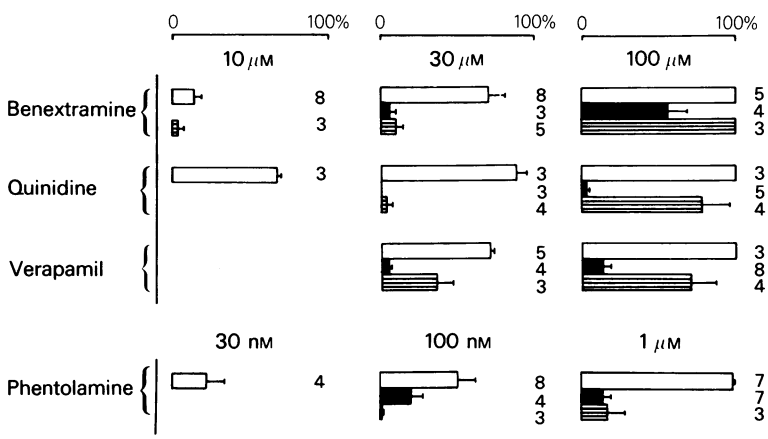

Figure 4 Inhibition (% vertical lines show s.e.) of adrenaline-(open columns), ADP-(solid columns), and collagen-induced platelet aggregation (lined columns) by benextramine, quinidine and verapamil (10–100 μM) and by phentolamine (0.03–1 μM). Numbers to the number of experiments.

platelet α-adrenoceptors. This difference has also been observed with the irreversible α-adrenoceptor blocking drug, phenoxybenzamine. Phenoxybenzamine was 100 times more potent than phentolamine as a postsynaptic α-adrenoceptor blocking drug in the cat isolated spleen and ten times more potent as a presynaptic α-adrenoceptor blocking drug in the cat spleen (Cubeddu, Barnes, Langer & Weiner, 1974) and the rabbit isolated heart (Starke, Montel & Schümann, 1971; Starke, Montel & Wagner, 1971) and had a very low potency in human platelets (Clayton & Cross, 1963; O'Brien, 1963; 1964; Bygdeman & Johnsen, 1969; Jakobs, Saur & Schultz, 1978; Lasch & Jakobs, 1979) or was inactive (Born, Mills & Roberts, 1967; Mills & Roberts, 1967a; Hsu, Knapp & Halushka, 1979). Compared to phentolamine, phenoxybenzamine had a higher affinity for both postsynaptic and presynaptic α-adrenoceptors but a much lower affinity for platelet α-adrenoceptors. Thus the two irreversible-α-adrenoceptor blocking drugs, benextramine and phenoxybenzamine, demonstrate that the platelet α-adrenoceptors differ from postsynaptic and presynaptic α-adrenoceptors.

Quinidine was a slightly more potent adrenaline antagonist than benextramine. The drug has postsynaptic α-adrenoceptor activity (Schmid, Nelson, Mark, Heistad & Abboud, 1974).

The potency of verapamil as an antagonist of the adrenaline-induced platelet aggregation was similar to that of benextramine. Verapamil is not known to antagonize adrenaline effects by adrenoceptor blockade.

Binding of benextramine by plasma proteins has not been studied; the possibility must be considered that plasma protein binding interferes with the assessment of the inhibitory potencies of the compounds under investigation.

The high concentrations of benextramine needed to antagonize the adrenaline effect, inhibited ADP- and collagen-induced aggregation. Similarly, high concentrations of phentolamine and phenoxybenzamine (100 μM) inhibit ADP-induced aggregation (Bygdeman & Johnsen, 1969), and 1 mM quinidine inhibits collagen and ADP-induced aggregation (Mason, Read, Saba & Shermer, 1972).

High concentrations of other drugs, such as certain local anaesthetics, histamine H_1-receptor antagonists and antidepressant drugs, inhibit ADP-induced platelet aggregation (O'Brien, 1962). Various histamine H_1-receptor antagonists and antidepressant drugs (10–50 μM) inhibit ADP, collagen and adrenaline-induced aggregation, the first phase of the adrenaline effect being inhibited less than the second phase (Mills & Roberts, 1967b). Verapamil inhibits adrenaline-induced, but not ADP-induced aggregation in a 50 μM concentration (Owen, Feinberg & Le Breton, 1980) and collagen-induced 5-hydroxytryptamine release from platelets in a 100 μM concentration (Haslam & Lynham, 1976).

In conclusion, benextramine antagonizes adrenaline-induced platelet aggregation in concentrations only slightly lower than those that inhibit ADP and collagen-induced aggregation. It differs from phentolamine which has a much higher α-adrenoceptor blocking potency in platelets.

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