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INHIBITION BY THE TETRAMINE DISULPHIDE, BENEXTRAMINE, OF CARDIAC CHRONOTROPIC HISTAMINE H_2-RECEPTOR-MEDIATED EFFECTS

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1 Benextramine (N,N'-bis[*o*-methoxybenzylamino)-*n*-hexyl]cystamine), which irreversibly blocks α-adrenoceptors and does not inhibit the H_1-receptor-mediated contractile effect of histamine on guinea-pig isolated ileum, also did not inhibit the H_1-receptor-mediated inotropic effect of histamine on guinea-pig isolated atrium.

2 Benextramine irreversibly inhibited the H_2-receptor-mediated chronotropic effect of histamine on guinea-pig isolated atrium.

3 Since its combination with the competitive H_2-receptor blocking drug cimetidine had an additive blocking effect, benextramine appears to act directly on the chronotropic H_2-receptor.

Introduction

Benextramine was introduced as an irreversible α-adrenoceptor blocking drug (Melchiorre, Yong, Benfey & Belleau, 1978). In contrast to the β-haloalkylamines which irreversibly block α-adrenoceptors and antagonize histamine effects on rabbit isolated aorta (Furchgott, 1954) and guinea-pig isolated ileum (Nickerson, 1956), benextramine does not inhibit the histamine-induced contraction of the rabbit aorta (Melchiorre *et al.*, 1978) or the guinea-pig ileum (Benfey, unpublished).

The smooth muscle stimulatory effect of histamine on guinea-pig ileum (Ash & Schild, 1966) and the inotropic effect of histamine on guinea-pig atrium (Reinhardt, Wagner & Schümann, 1974; Steinberg & Holland, 1975) are mediated by H_1-receptors since they are blocked by H_1-receptor antagonists and not by H_2-receptor antagonists. Therefore, it was not surprising to find that benextramine does not inhibit the inotropic effect of histamine on guinea-pig atrium.

The chronotropic effect of histamine on guinea-pig atrium is not inhibited by H_1-receptor antagonists (Trendelenburg, 1960) but is inhibited by H_2-receptor antagonists (Black, Duncan, Durant, Ganellin & Parsons, 1972). We have found that benextramine inhibits the chronotropic effect of histamine on the guinea-pig atrium.

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Methods

Spontaneously beating atria (Benfey & Greeff, 1961) and strips of left atria from immature guinea-pigs (150 to 200 g weight) were suspended at 31°C in a solution of the following composition (mM): NaCl 114.9, $NaHCO_3$ 24.9, KCl 4.7, $CaCl_2$ 1.8, KH_2PO_4 1.2, $MgSO_4$ 1.2 and glucose 10, which was aerated with 5% CO_2 in O_2. The left atria were driven with square-wave pulses of 1 ms duration at a voltage slightly above threshold (2–4 V) through platinum electrodes by a Grass stimulator at a rate of 1 Hz. Isometric contractions were recorded by a Grass FT 03B force-displacement transducer and a Grass polygraph. Diastolic tension was about 0.5 g. Dose-response curves of histamine were established cumulatively. An atrial preparation was used for only one dose-response experiment.

Incubation with benextramine was for 1 h. Histamine dose-response curves were determined either in the presence of benextramine or 1 h after removal of the drug. During the hour after the withdrawal of benextramine the bath fluid was repeatedly changed. Incubation with cimetidine was for 1 or 2 h, and the histamine dose-response experiments were always carried out in the presence of the drug.

The EC_{50} value was determined by fitting a regression line by the least squares method through the linear portion of the dose-response curve. The dose-ratio was calculated as the EC_{50} in the presence of antagonist and in its absence. The potency of the competitive H_2-receptor blocking drug cimetidine was calculated as K_B = [cimetidine]/dose-ratio − 1.

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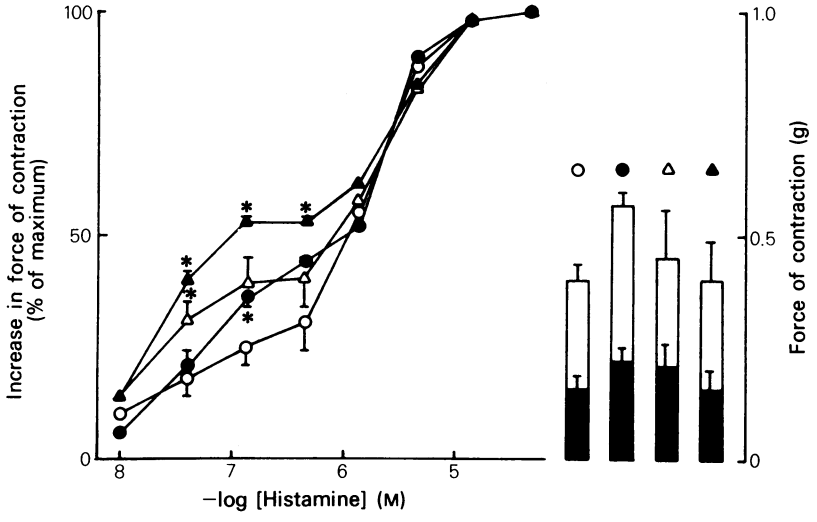

Figure 1 Inotropic effect of histamine on guinea-pig left atrium in the absence (○) and presence of 3 μM cimetidine (●), 3 μM benextramine (△), and 10 μM benextramine (▲). Means of 4–6 experiments; verticle lines indicate s.e. The solid columns show control force of contraction and the open columns maximal force of contraction. *Different from control ($P < 0.05$).

Benextramine was combined with cimetidine in order to see whether the two drugs act at the same site. Competition between antagonists can be investigated by measuring the agonist dose-ratio produced by two antagonists applied simultaneously. If two antagonists, which individually result in agonist dose-ratios DR_1 and DR_2 respectively, are applied simultaneously, the resulting combined dose-ratio $DR_{1+2} = DR_1 + DR_2 - 1$ if they compete (Paton & Rang, 1965).

Statistical calculations were done according to conventional procedures (Snedecor & Cochran, 1967).

The drugs were benextramine tetrahydrochloride monohydrate (BHC; N,N′-bis[6-(*o*-methoxybenzylamino)-*n*-hexyl]cystamine; Melchiorre *et al.*, 1978; Aldrich), cimetidine hydrochloride (Smith, Kline & French), and histamine dihydrochloride (Hoffmann-La Roche).

Results

Figure 1 shows that benextramine or cimetidine did not inhibit the inotropic effect of histamine on the guinea-pig left atrium but that 3 and 10 μM benextramine and 3 μM cimetidine increased the effect of low concentrations of histamine.

Benextramine inhibited the chronotropic effect of histamine on the guinea-pig right atrium. The log concentration-response curve of histamine was shifted along the concentration axis and its maximum was depressed (Figure 2). The histamine blockade by benextramine persisted for 1 h after the drug had been withdrawn (Figure 3).

Table 1 shows the dose-ratios produced by benextramine and cimetidine alone and in combination. The K_B of cimetidine was 0.35 ± 0.047 μM (mean ± s.e. of 8 experiments) after an incubation of 1 h and 0.64 ± 0.16 μM (mean ± s.e. of 9 experiments) after an incubation of 2 h. The potency of benextramine was slightly lower than that of cimetidine.

The experimental combination dose-ratios of benextramine and cimetidine were not significantly different from the expected combination dose-ratios ($P > 0.05$; Table 1). It thus appears that the site of action of the two drugs is the same.

Discussion

Benextramine is an irreversible α-adrenoceptor blocking drug in the rabbit isolated aorta; its potency is about ten times lower than that of phentolamine (Melchiorre *et al.*, 1978), and it inhibits the positive inotropic effect of phenylephrine in the rabbit isolated atrium with an IC_{50} of 0.26 μM (Benfey, Belleau, Brasili, Giannella & Melchiorre, 1980). Lippert & Belleau (1973) proposed that a tetramine disulphide acts with its four symmetrical cationic charges on complementary negative charges on the α-adrenoceptor and induces a conformational change that leads to the unmasking of a buried thiol which then participates in a disulphide interchange reaction with the drug. The resulting disulphide bond between

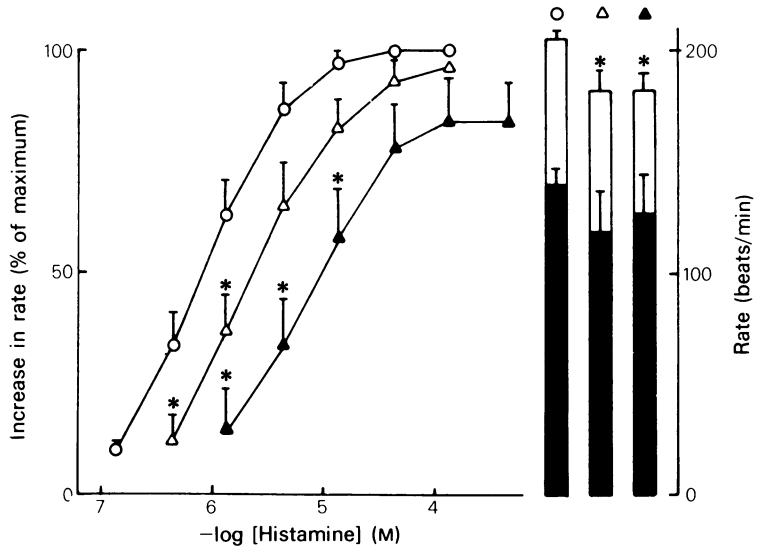

Figure 2 Chronotropic effect of histamine on guinea-pig right atrium in the absence (○) and presence of 3 μM benextramine (△) and 10 μM benextramine (▲). Means of 4–6 experiments; vertical lines indicate s.e. The solid columns show control rate and the open columns maximal rate. *Different from control ($P<0.05$).

drug and receptor appears to sit below the receptor surface because it is refractory to the reducing action of mercaptoethanol and only the cationic thiol cysteamine can reverse the blockade.

Benextramine inhibits the chronotropic effect of histamine on the guinea-pig atrium but not the inotropic effect on the paced left atrium and thus acts like the H_2-receptor antagonists, burimamide (Reinhardt *et al.*, 1974; Reinhardt, Schmidt, Brodde & Schümann, 1977) and metiamide (Steinberg & Hol-

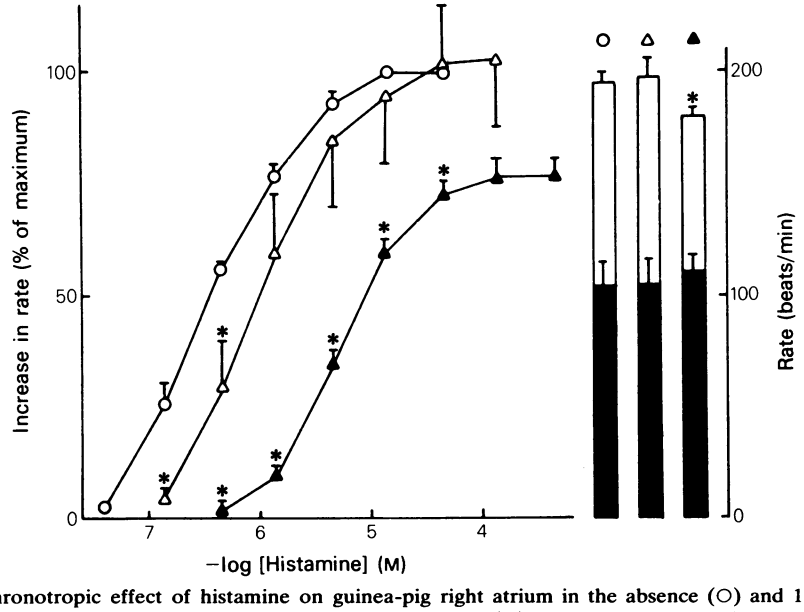

Figure 3 Chronotropic effect of histamine on guinea-pig right atrium in the absence (○) and 1 h after a 1 h incubation with 3 μM benextramine (△) and 10 μM benextramine (▲). Means of 4–6 experiments; vertical lines indicate s.e. The solid columns show control rate and the open columns maximal rate. *Different from control ($P<0.05$).

Table 1 Histamine dose-ratios produced by benextramine and cimetidine alone and in combination

Incubation time (h)	*Benextramine concentration* (μM)	*Benextramine dose-ratio* ($DR_1 \pm$ s.e.)	*Cimetidine concentration* (μM)	*Cimetidine dose-ratio* ($DR_2 \pm$ s.e.)	*Experimental combination dose-ratio* ($DR_{1+2} \pm$ s.e.)	*Expected combination dose-ratio* ($DR_1 + DR_2 - 1$)
1*	1	1.9 ± 0.41 (3)†				
1	3	3.0 ± 0.85 (5)	1	3.6 ± 1.2 (4)	6.0 ± 1.6 (4)	5.6
1	3	3.0 ± 0.85 (5)	3	7.8 ± 0.30 (4)	11 ± 2.4 (4)	9.8
1	10	10 ± 4.1 (4)	1	3.6 ± 1.2 (4)	10 ± 1.7 (4)	13
1	10	10 ± 4.1 (4)	3	7.8 ± 0.30 (4)	16 ± 5.0 (3)	17
2	1	2.7 ± 0.63 (4)				
2	3	5.3 ± 0.66 (4)	1	2.7 ± 0.80 (4)	6.7 ± 0.60 (4)	7.0
2	3	5.3 ± 0.66 (4)	3	5.6 ± 1.2 (5)	14 ± 2.3 (5)	9.9
2	10	14 ± 2.7 (4)	1	2.7 ± 0.80 (4)	16 ± 4.5 (4)	16
2	10	14 ± 2.7 (4)	3	5.6 ± 1.2 (5)	21 ± 5.4 (5)	19

*Incubation time 1 h: both benextramine and cimetidine present; incubation time 2 h: benextramine withdrawn after 1 h, bath fluid repeatedly changed, cimetidine present.
†Number of experiments in parentheses.

land, 1975). Like metiamide (Steinberg & Holland, 1975; Wilson & Broadley, 1980) it slightly potentiates the inotropic effect of histamine on the guinea-pig left atrium (Figure 1).

The competitive H_2-receptor blocking drug, cimetidine, antagonizes chronotropic effects of histamine with K_B values ranging between 0.21 μM and 0.79 μM (Brimblecombe, Duncan, Durant, Ganellin, Parsons & Black, 1975; Bradshaw, Brittain, Clitherow, Daly, Jack, Price & Stables, 1979; Yellin, Buck, Gilman, Jones & Wardleworth, 1979; McCulloch, Medgett & Rand, 1979). Thus our K_B values, 0.35 μM and 0.64 μM, do not differ from the published values.

Unlike cimetidine, benextramine not only shifted the histamine log dose-response curve but also depressed its maximum. The same occurred when benextramine antagonized the noradrenaline effect in the rabbit aorta (Melchiorre *et al.*, 1978) and the phenylephrine effect in the rabbit atrium (Benfey *et al.*, 1980), and when the β-haloalkylamine dibenamine antagonized the adrenaline effect in the rabbit aorta (Furchgott, 1955). Dibenamine also is an irreversible α-adrenoceptor blocking drug. Since its combination with cimetidine had an additive blocking effect, it appears that benextramine acts directly on the H_2-receptor. And since its effect was irreversible, benextramine presumably forms a covalent bond with the H_2-receptor in the guinea-pig heart as it does with the α-adrenoceptor.

The β-haloalkylamine phenoxybenzamine blocks α-adrenoceptors and H_1-receptors irreversibly, and it is not clear whether it also blocks H_2-receptors. Cook & Krueger (1978) found that phenoxybenzamine does not inhibit the H_2-receptor-mediated inotropic effect of histamine in the guinea-pig right ventricle, but Johnson (1979) found that phenoxybenzamine inhibits the stimulation of adenylate cyclase activity caused by histamine in the guinea-pig ventricle, and H_2-receptor stimulation, but not H_1-receptor stimulation, increases cardiac adenylate cyclase activity (McNeill, 1980).

Other drugs have been found to act on both α-adrenoceptors and H_2-receptors. Thus imidazole derivatives with H_2-receptor blocking activity inhibit α-adrenoceptor mediated effects. Burimamide competitively antagonized the adrenaline effect on the rat seminal vesicle (Brimblecombe, Duncan, Owen & Parsons, 1976) and the noradrenaline effect on the rabbit aorta and reversed the clonidine effect on the electrically induced noradrenaline release in the guinea-pig atrium (McCulloch *et al.*, 1979). Metiamide antagonized the presynaptic α-adrenoceptor-mediated effect of noradrenaline and clonidine on the electrically induced twitch response and the noradrenaline release in the mouse vas deferens (Griffith, Marshall & Nasmyth, 1978). Cimetidine inhibited the contraction of the rabbit aorta caused by noradrenaline and the presynaptic effect of clonidine on noradrenaline release in the guinea-pig atrium (McCulloch *et al.*, 1979). Some imidazoline derivatives stimulate α-adrenoceptors and H_2-receptors. Thus clonidine is a partial agonist on α-adrenoceptors in rabbit papillary muscle (Schümann & Endoh, 1976) and rat vas deferens (Malta, Ong, Raper, Tawa & Vaughan, 1980) and on cardiac presynaptic α-adrenoceptors (Werner, Starke & Schümann, 1972). The inotropic effect of clonidine on the guinea-pig isolated heart is mediated by H_2-receptors (Czongrady & Kobinger, 1974), and clonidine is a full agonist on inotropic H_2-receptors in guinea-pig right ventricle (Malta *et al.*, 1980) and a

partial agonist on chronotropic H_2-receptors in guinea-pig atrium (McCulloch *et al.*, 1979; Medgett & McCulloch, 1980). Tolazoline and tetrahydrozoline are partial agonists on α-adrenoceptors in rabbit aorta, chronotropic H_2-receptors in guinea-pig atrium (Sanders, Miller & Patil, 1975), and inotropic and chronotropic H_2-receptors in guinea-pig isolated heart (Zavecz & Yellin, 1981).

We conclude that common features of the α-adrenoceptor and the H_2-receptor permit a tetramine disulphide and certain imidazoles and imidazolines to act on both receptors.

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