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Stereospecific blockade of α_2-adrenoceptors by (+)-butaclamol: implications for the characterization of dopamine receptors

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The stereospecific binding of (+)-butaclamol to cell membranes is a useful tool in the characterization of dopamine receptors as the contribution of non-specific binding to cell membranes can be assessed using the inactive (−)-isomer (see Seeman 1977). On this basis, (+)-butaclamol has been recently used to define dopamine receptors in the renal (Schmidt & Imbs 1980) and mesenteric (Brodde & Gross 1980; Brodde et al 1981a, b) vascular beds. However, many dopamine receptor antagonists are potent α-adrenoceptor antagonists showing a variable degree of selectivity for α_1- or α_2-adrenoceptors (Spedding 1980; Petersen 1981). We have therefore examined the isomers of butaclamol for α-adrenoceptor antagonist activity using phenylephrine-induced contractions of rat aorta to assess effects on α_1-adrenoceptors and clonidine-induced inhibition of the responses to field stimulation of guinea-pig ileum to assess effects on α_2-adrenoceptors.

Ileum preparations from male guinea-pigs (300–550 g) and spirally cut (25 mm × 3 mm) aorta preparations from male Sprague-Dawley rats (230–400 g) were set up in 10 ml isolated organ baths containing Tyrode solution of the following composition (mM): NaCl 137, KCl 2·7, $MgCl_2$ 1·1, $CaCl_2$ 1·8, $NaHCO_3$ 11·9, NaH_2PO_4 0·4, glucose 5·5 and (±)-propranolol 0·003. The solution was maintained at 35 ± 1 °C and gassed with a mixture of 95% O_2 and 5% CO_2. Resting tension was set at 200–300 mg for the ileum preparations and 500 mg for the aorta. Responses were recorded isometrically. The ileum preparations were continuously stimulated at 0·05 Hz (1 ms pulse duration, supramaximal voltage) via platinum ring electrodes placed above and below the tissue. Cumulative concentration-response curves were obtained by adding agonists to the bathing solution using logarithmic dosage increments (van Rossum 1963). A 30 min washout period was allowed between each curve and the antagonists were incubated for 20 min between curves. EC 50 values were calculated from the curves and dose ratios were estimated as the ratio of the EC 50 in the presence of the antagonist compared with the EC 50 obtained in the first curve. There was no significant change in the sensitivity of the preparations to the agonists with time during the course of the experiments. pA_2 values were calculated as the intercept of the plot of log (dose ratio-1) against log molar concentration of the antagonist (Arunlakshana & Schild 1959).

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The inhibitory effects of clonidine on the contractile responses to field stimulation of guinea pig ileum were antagonized by yohimbine (1 μM, dose ratio > 300), but not by prazosin (0·1 μM, dose ratio < 2) or cimetidine (30 μM, dose ratio < 2). (+)-Butaclamol (0·03–10 μM) displaced cumulative concentration response curves to clonidine to the right, whereas (−)-butaclamol was ineffective (Fig. 1).

The antagonism was selective because (+)-butaclamol (10 μM) did not antagonize the inhibitory effects of adenosine (0·1–10 μM) on the responses to field stimulation. Furthermore, the isomers of butaclamol had little direct effect on the responses to field stimulation; only at the highest concentration tested (10 μM) was (20–30%) inhibition observed.

The pA_2 value for the antagonism of clonidine by (+)-butaclamol calculated from these curves was 8·0 ± 0·1 and the slope of the Arunlakshana & Schild (1959) plot was −1·11 ± 0·08. Similar pA_2 values were obtained when α-methylnoradrenaline (8·2 ± 0·3, slope −1·21 ± 0·11, n = 4) or dopamine (7·6 ± 0·2, slope −1·6 ± 0·15, n = 4) were used as agonists. The pA_2 values for yohimbine as an antagonist of α-methylnoradrenaline and of dopamine were 8·1 ± 0·2 (slope −1·43 ± 0·37, n = 4) and 7·9 ± 0·2 (slope −1·15 ± 0·10, n = 4) respectively, indicating antagonism of α_2-adrenoceptors (Drew 1978; Doxey & Roach 1980).

Phenylephrine-induced contractions of rat aorta were antagonized by prazosin (0·01 μM, dose ratio > 300) and by (+)-butaclamol (0·1–10 μM, Fig. 1). (−)-Butaclamol (10 μM) also antagonized responses to phenylephrine, thus some of the antagonist effects of

Table 1. Antagonist potencies of drugs at α_2-adrenoceptors and at dopamine receptors in the renal and mesenteric vasculature.

Antagonist	pA_2 α_2-receptors (ileum)	pA_2 Dopamine vascular receptor
(+)-Butaclamol	8·0[1]	8·7[2], 6·8[3]
Haloperidol	< 6·5[1]	6·1[2]
Metoclopramide	5·6[1]	5·2[4]
Sulpiride	5·1[1]	4·8[2]

[1] Data from this study or recalculated from Spedding (1981).
[2] Schmidt & Imbs (1980).
[3] Brodde et al (1981a).
[4] Brodde et al (1981b).

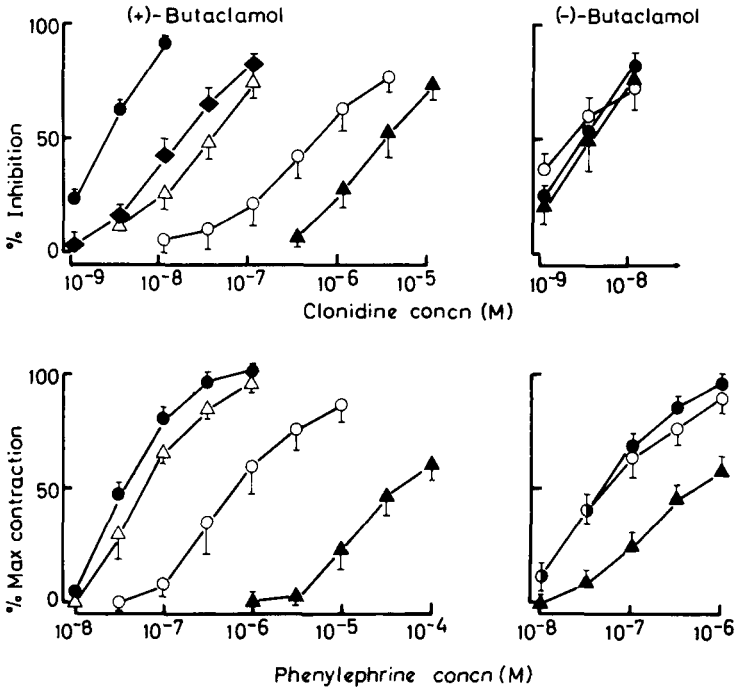

FIG. 1. The effects of the isomers of butaclamol (● - ● control; ◆—◆ 0·03 μM; △ - △ 0·1 μM; ○ - ○ 1 μM; ▲ - ▲ 10 μM) on the inhibitory effects of clonidine on the contractile responses to field stimulation of guinea-pig ileum (upper panels) and on phenylephrine-induced contractions of rat aorta (lower panels). Vertical bars represent s.e. mean, n = 5–6. There were no time dependent changes in the sensitivity of the tissues during the course of these experiments.

high concentrations of (+)-butaclamol may have included a non-specific component. The apparent pA_2 for (+)-butaclamol in these experiments was 7·0 ± 0·1 (slope −1·17 ± 0·10), confirming previous reports that the drug is an antagonist at α-adrenoceptors (Voith & Herr 1975; Seeman 1977). However (+)-butaclamol was significantly ($P < 0·001$) less potent as an antagonist of phenylephrine-induced contractions in the rat aorta compared with its potency as an antagonist of clonidine in the guinea-pig ileum, indicating a degree of selectivity for α_2-adrenoceptors.

The finding that (+)-butaclamol had equivalent potency to yohimbine as an antagonist of α_2-adrenoceptors constitutes further evidence that some forms of dopamine receptor have structural similarities to α_2-adrenoceptors (Spedding 1980). In this regard, the in vitro potencies of several antagonists which have been used to define the dopamine vascular receptor are similar to their potencies at α_2-adrenoceptors (Table 1). These receptors can, however, be differentiated pharmacologically in several ways. Thus, spiroperidol is a potent ligand at the dopamine vascular receptor (Brodde & Gross 1980) but is not a potent antagonist at α_2-adrenoceptors ($pA_2 < 7$, unpublished observation) and yohimbine, although an antagonist at certain dopamine receptors in the brain (Scatton et al 1980), does not antagonize the dopamine vascular receptor (Listinsky et al 1980). Furthermore, clonidine is not a potent ligand at the dopamine vascular receptor, as defined by inhibition of spiroperidol binding (Brodde & Gross 1980).

Nevertheless, the structural *similarities* between dopamine vascular receptors and α_2-adrenoceptors may have important physiological implications (Listinsky et al 1980), since both types of receptor are located in the kidneys of some species (Young & Kuhar 1980; McPherson & Summers 1981), and dopamine is an agonist at both receptors (e.g. this study; Baggio & Ferrari 1981; Schmidt & Imbs 1980). As activation of both types of receptor results in diuresis in the rat (Pendleton & Sherman 1976; Baggio & Ferrari 1981) whereas renal blood flow is either decreased (α_2-adrenoceptor, Imbs, Schmidt & Spedding, unpublished observation) or increased (dopamine receptor), the definition of the renal effects of dopamine is critically dependent upon the specificity of the antagonists used. The present finding that (+)-butaclamol antagonizes α_2-adrenoceptors at similar concentrations to those blocking dopamine receptors suggests caution in the use of the compound for this purpose.

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Selectivity of cyproheptadine as assessed by radioligand binding

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Cyproheptadine is widely used experimentally as an anti-5-hydroxytrytaminergic, antihistaminic and anti-acetylcholine drug, these intrinsic properties being well established (Stone et al 1961). More recently, studies on cyproheptadine inhibition of insulin secretion (Donatsch et al 1980) have led to the suggestion that the drug interacts with calcium channels and in so doing produces its in vivo pharmacological effects. Examination of cyproheptadine in radioligand receptor-binding assays showed that it was some five orders of magnitude more active in displacing appropriate radioligands from central histamine, 5-HT-2 and muscarinic cholinergic receptor sites than in inhibiting depolarization dependent calcium fluxes (Donatsch et al 1980).

Radioligand binding to central receptors was measured using membranes prepared from rat brain by described methods as follows (with final radioligand concentration and tissue preparation) muscarinic cholinergic ([^{3}H] quinuclidinyl benzilate ([^{3}H]QNB); 60 pM; rat cortical S_1 fraction; Yamamura & Snyder 1974); α-adrenoceptor ([^{3}H]WB 4101; 0·2 nM; rat forebrain; Greenberg et al 1976); β-adrenoceptor ([^{3}H]-dihydroalprenolol ([^{3}H]DHA); 1·0 nM; rat cortical P_2 fraction; Bylund & Snyder 1976); dopamine ([^{3}H]-apomorphine; 0·2 nM; rat caudate; Seeman et al 1976); neuroleptic ([^{3}H]spiroperidol; 0·1 nM; rat caudate; Burt et al 1976); 5-HT-1 ([^{3}H]5-HT; 4 nM; rat forebrain; Bennett & Snyder 1976); 5-HT-2 ([^{3}H]mianserin; rat forebrain; 0·75 nM; Peroutka & Snyder 1981); 5-HT-1 and 2 ([^{3}H]lysergic acid diethylamide (LSD); 2 nM; rat forebrain; Bennett & Snyder 1976); GABA ([^{3}H]GABA; 10 nM; whole brain crude synaptic membranes; Enna & Snyder 1977); anxiolytic ([^{3}H]diazepam; 1·5 nM; rat cortical P_2 fraction; Squires & Braestrup 1977); adenosine A-1; ([^{3}H]2-chloroadenosine (2-CADO); 1·0 nM; whole brain crude synaptic membranes; Williams & Risley 1980).

Examination of cyproheptadine in eleven receptor binding assays (Table 1), confirmed the acetylcholine-like and 5-HT-ergic properties of the molecule; the compound was approximately equiactive in displacing [^{3}H]QNB from muscarinic cholinergic sites and [^{3}H]-mianserin from 5-HT-2 sites with K_i's of 3–6 nM. Cyproheptadine was about sixty times less active at central 5-HT-1 sites ([^{3}H]5-HT binding) than at 5-HT-2 sites ([^{3}H]mianserin binding). Cyproheptadine also displaced [^{3}H]apomorphine and [^{3}H]spiroperidol from dopaminergic binding sites with about the same efficacy ($K_i \simeq 100$ nM) and had some α-adrenergic activity as evidenced by the displacement of [^{3}H]WB 4101 (K_i = 178 nM). No significant β-adrenergic, GABAenergic, anxiolytic or adenosine A-1-related activity was observed (Table 1). The histaminergic activity of cyproheptadine, while not measured in the present study, has been demonstrated in vitro by Peroutka & Snyder (1981) who found an IC50 of 5·8 nM (K_i = 4·7 nM) using radioligand binding.

It seems unlikely therefore that the demonstrated in vivo pharmacological activity of cyproheptadine (Stone et al 1961) can be ascribed to calcium channel blockade (Donatsch et al 1980).

The finding that cyproheptadine was approximately equiactive in the 5-HT-2, histamine and muscarinic cholinergic radioligand assays indicates that these latter

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