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The *in vitro* pharmacology of xamoterol (ICI 118,587)

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1 The effect of xamoterol and (−)-isoprenaline have been compared for their activity at β-adrenoceptor sites in a number of *in vitro* cardiac and smooth muscle preparations.

2 Xamoterol produced weak positive chronotropic effects in guinea-pig, rat and cat atria (intrinsic activity <0.55, (−)-isoprenaline = 1). Positive inotropic effects were obtained in driven left atria of the cat but were absent in guinea-pig left atrial and right ventricular strip preparations. Agonistic effects were due to β_1-adrenoceptor stimulation.

3 Xamoterol was without β-adrenoceptor-mediated inhibitory effects in guinea-pig ileal, tracheal and uterine preparations and in the rat vas deferens and oestrogen-primed uterus. Weak β_2-adrenoceptor-mediated relaxation was obtained in progesterone-primed rat uteri.

4 Xamoterol produced non-specific inhibitory effects in guinea-pig ileal and tracheal preparations.

5 Xamoterol acted as a competitive antagonist at β_1- (pA_2 range = 7.4 to 7.8) and β_2-adrenoceptors (pA_2 range 5.2 to 6.2) and displaced [^{125}I]-iodocyanopindolol from guinea-pig left atrial ($pK_D = 7.25$) and uterine (pK_D 5.24) membrane preparations.

6 It is concluded that xamoterol displays a selective affinity for β_1-adrenoceptors. Although its partial agonistic actions are more evident at β_1-adrenoceptor sites, like prenalterol, xamoterol displays a degree of tissue rather than receptor-dependent selectivity.

Introduction

In 1981, Barlow *et al.* reported on the synthesis and pharmacological actions of a number of arylethanolamine- and aryloxypropanolamine-based cardiac stimulants. One compound (ICI 118,587, xamoterol, Corwin, Figure 1) was selected for more detailed studies on the basis that, (i) it raised the heart rate of anaesthetized, syrosingopine-treated dogs to 50% of the isoprenaline maximum, (ii) it was orally active and (iii) it did not elicit vasodilatation in the hind-limb in doses up to 250 times its cardiac ED_{50} value. A later study (Nuttall & Snow, 1982), in addition to describing the positive inotropic actions of xamoterol, found it to be 13 times more potent as an antagonist at cardiac as opposed to vascular β-adrenoceptor sites in dogs. Thus, on the basis of these *in vivo* results, xamoterol was classed as a partial agonist with selective β_1-adrenoceptor actions. Preliminary observations in man support this classification, since xamoterol increases resting heart rate but reduces the elevated heart rate observed during exercise (Marlow *et al.*, 1980).

To date, the pharmacology of xamoterol has been established by use of *in vivo* techniques. The present study was therefore undertaken to investigate the actions of xamoterol at β-adrenoceptor sites in a number of *in vitro* preparations.

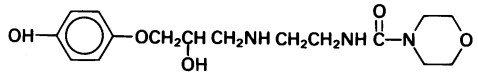

Figure 1 Structure of xamoterol (ICI 118,587).

Methods

The tissues used in the present experiments were, unless otherwise stated, taken from reserpine-pretreated (1 mg kg^{-1}, i.p. 18 h) animals and set up under a resting tension of 0.5 g in organ baths containing Krebs solution (NaCl 6.9, KCl 0.4, $MgSO_4.7H_2O$ 0.14, $NaHCO_3$ 2.1, dextrose 2.0, $CaCl_2.2H_2O$ 0.28, $NaH_2PO_4.2H_2O$ 0.15 g l^{-1}) maintained at 37°C and aerated with 5% CO_2 in O_2. Ascorbic acid (0.02 g l^{-1}) was added to the physiological salt solution to reduce oxidation of the catecholamines. Extraneuronal and neuronal uptake, and possible α-adrenoceptor mediated effects, were prevented using

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either phenoxybenzamine (50 μmol l^{-1} for 30 min followed by 6 washes over 30 min), or a combination of cocaine (30 μmol l^{-1}), hydrocortisone (50 μmol l^{-1}) and phentolamine (10 μmol l^{-1}).

Changes in tension were recorded using Grass FT03c force displacement transducers, traces being displayed on Grass 79B recorders.

Cardiac preparations

Positive chronotropic responses were assessed in spontaneously beating right atrial preparations from cats and guinea-pigs and in whole atria from rats. Contractions were used to trigger a Grass 7P4F tachograph. Inotropic activity was monitored in electrically driven cat (2 Hz) and guinea-pig (4 Hz) left atrial and guinea-pig right ventricular strips (1 Hz). Driving stimuli were delivered to the tissues from a Grass S6 stimulator via a platinum punctate electrode using square wave pulses of 2.5 ms duration at twice the threshold voltage required to drive the preparations (for further details see Iakovidis *et al.*, 1980).

In the above experiments, atria from non-reserpine-treated cats were used, since in pilot experiments it was frequently found that the combination of reserpine pretreatment and uptake blockade with phenoxybenzamine resulted in unstable preparations with depressed contractility. Rat atria were set up at 32°C under 0.2 g tension and a lower concentration of phenoxybenzamine (1 μmol l^{-1}) was used for uptake blockade (Mattsson *et al.*, 1982).

In further experiments, changes in effective refractory period were monitored using a paired pulse technique in guinea-pig left atria driven at 4 Hz (Mylecharane & Raper, 1971). The pulse interval was increased from an initial 10 ms until potentiated contractions were observed, and this pulse interval was taken as a measure of effective refractory period. Drugs were administered cumulatively and measurements made after a 15 min contact period with each concentration used.

Smooth muscle preparations

Relaxant responses were obtained in tracheal strips (Iakovidis *et al.*, 1980) in which tone was induced with carbachol (0.5 μmol l^{-1}) and in guinea-pig and rat uterine preparations in which a K^+-depolarizing solution was used (Krstew *et al.*, 1982; Mattsson *et al.*, 1982; Mattsson *et al.*, 1983). The uterine preparations were taken from animals pretreated for 24 h with stilboestrol (guinea-pigs 0.1 mg kg^{-1}, rats 1 mg kg^{-1}, i.p.) or with progesterone (rats 10 mg kg^{-1} i.p. daily for 4 days).

In guinea-pig ileal and in rat vas deferens preparations inhibitory activity was assessed in terms of reductions in contractions elicited by electrical stimulation. Ileal preparations were stimulated transmurally at 0.1 Hz using square wave pulses of 2.5 ms duration and supramaximal voltage (Malta *et al.*, 1981) and rat vas deferens preparations at 0.3 Hz via concentric ring electrodes (3 ms, supramaximal voltage, Krstew *et al.*, 1982).

Expression of results

In all studies, consistent cumulative concentration-effect curves to (−)-isoprenaline were first obtained and thereafter a cumulative curve to xamoterol was established. Agonistic responses were expressed as a percentage of the maximal response to (−)-isoprenaline and relative activities (EC_{50} xamoterol: EC_{50} (−)-isoprenaline) and intrinsic activities (α, (−)-isoprenaline = 1) calculated. Additional experiments were performed in which maximal agonist concentrations of xamoterol found in the cumulative curve experiments were added to the bath as single bolus doses ($n = 2$ for each preparation).

In both cardiac and smooth muscle preparations, agonist actions of xamoterol were either weak or absent. It was therefore possible to assess the β-adrenoceptor antagonistic effects of xamoterol from rightward shifts of superimposed concentration-effect curves to (−)-isoprenaline or other agonists using the method described by Malta & Raper (1974). A 45 min contact time was allowed for each concentration of xamoterol used, and pA_2 values and the slopes of the relationship between log (dose-ratio − 1) and log (molar xamoterol concentration) calculated (Arunlakshana & Schild, 1959). The range of dose-ratios used to assess antagonism was 10–7600 for atrial, 2–70 for tracheal, 10–510 for uterine and 7–900 for vas deferens preparations. Negative log K_B values (pK_B, Furchgott, 1972) were calculated in experiments with guinea-pig ileal preparations since only one concentration of xamoterol was used in each experiment.

Radioligand binding studies

Left atrial membranes (1:300 vol), and uterine membranes (1:100 vol) from stilboestrol pretreated guinea-pigs (0.1 mg kg^{-1} i.p. 24 h) were prepared in a Krebs phosphate buffer (pH 7.4) as previously described (McPherson *et al.*, 1984).

The radioligand used was (−)-[^{125}I]-iodocyanopindolol ([^{125}I]-CYP, approximately 2000 Ci $mmol^{-1}$, Amersham). Drug displacement studies were performed by combining 150 μl of homogenate with 50 μl of radioligand (final concentration 50–80 pmol l^{-1}) in a total volume of 250 μl Krebs phosphate buffer containing (final concentration) 0.1 mmol l^{-1} GTP, 1 mmol l^{-1} ascorbic acid and 0.1 mmol l^{-1} EDTA. Non-specific binding was defined as that found in the presence of propranolol 1 μmol l^{-1}. Incubation of

tubes was carried out at 37°C for 70 min and terminated by the addition of 8 ml ice-cold buffer followed by rapid filtration through Whatman GF/B filters. Radioactivity retained on the filters was measured with a Packard Gamma Counter (Model 5320) at an efficiency of approximately 46%. All assays were performed in triplicate. The binding data were analyzed by two computer programmes EBDA (McPherson, 1983a;b) which performed preliminary Hofstee analysis and created a file for the second programme, LIGAND (Munson & Rodbard, 1980). The latter was used to obtain final parameter estimates.

Drugs used

The drugs used were (−)-isoprenaline bitartrate (Wyeth); fenoterol hydrobromide (Boehringer Ingelheim); reserpine (Serpasil) and phentolamine hydrochloride (Ciba-Geigy); xamoterol hemifumarate, atenolol, propranolol hydrochloride and ICI 118,551 hydrochloride (erythro-DL-1 (7-methylindan-4-yloxy) -3-isopropylaminobutan-2-ol) (Imperial Chemical Industries); phenoxybenzamine hydrochloride (Smith, Kline & French), cocaine hydrochloride (Macfarlane Smith), disodium stilboestrol diphosphate (Honvan, Bristol), hydrocortisone sodium succinate (Glaxo), histamine dihydrochloride, noradrenaline bitartrate, carbachol chloride, acetylcholine chloride and progesterone (Sigma) and calcium chloride dihydrate (Ajax Chemicals).

Stock solutions (10 mmol l^{-1}) of the drugs were prepared in either 10 mmol l^{-1} hydrochloric acid or distilled water and dilutions were made using the appropriate physiological salt solutions containing ascorbic acid (0.02 g l^{-1}). In uterine preparations, drugs were diluted using the K^+-depolarizing solution. Stock solutions of phenoxybenzamine were prepared using 95% ethanol containing 1 µl of 10 mol l^{-1} HCl in each millilitre of diluent.

Results

Cardiac preparations

(−)-Isoprenaline and xamoterol produced concentration-dependent positive chronotropic effects in right atrial preparations from cats, guinea-pigs and rats (Table 1). In these experiments mean intrinsic activities ranged from 0.16 to 0.55 and relative activities of approximately 1, 9 and 25 were obtained in the cat, guinea-pig and rat respectively. Figure 2 shows mean concentration-effect curves for (−)-isoprenaline and xamoterol in rat atrial preparations. Once established, the effects of xamoterol were poorly reversed by washing, hence testing its agonistic actions both before and after β-adrenoceptor blockade was difficult. However, in the continuing presence of concentrations of xamoterol which produced maximal chronotropic effects, the selective β_1-adrenoceptor antagonist atenolol (2 µmol l^{-1}) reversed the increases in atrial rate while the selective β_2-adrenoceptor antagonist, ICI 118,551, was without effect at a concentration of 50 nmol l^{-1}.

The greater potency of xamoterol in cat as opposed

Table 1 β-Adrenoceptor agonist activity of (−)-isoprenaline and xamoterol in isolated tissue preparations

			pD_2			
Tissue	*Response*	n	*Isoprenaline*	*Xamoterol*	α	*RA*
Guinea-pig						
Right atria	Rate	5	8.30 ± 0.03	7.57 ± 0.22	0.16 ± 0.02	9.06 ± 4.35
Left atria	Force	4	8.23 ± 0.10		No effect	
Right ventricle	Force	4	7.50 ± 0.04		No effect	
Ileum	Inhibition	4	7.99 ± 0.11		Non-β-receptor mediated inhibition	
Trachea	Relaxation	6	8.19 ± 0.02		Non-β-receptor mediated relaxation	
Uterus (oestrogen)	Relaxation	4	8.74 ± 0.16		No effect	
Cat						
Right atria	Rate	5	8.88 ± 0.10	9.08 ± 0.33	0.31 ± 0.07	1.24 ± 0.70
Left atria	Force	7	8.88 ± 0.08	8.18 ± 0.37	0.21 ± 0.06	9.62 ± 5.00
Rat						
Atria	Rate	4	9.03 ± 0.17	7.66 ± 0.18	0.55 ± 0.07	24.94 ± 4.34
Uterus (oestrogen)	Relaxation	4	8.94 ± 0.06		No effect	
Uterus (progesterone)	Relaxation	4	9.08 ± 0.15	6.68 ± 0.09	0.35 ± 0.05	377 ± 182
Vas deferens	Inhibition	7	7.30 ± 0.04		No effect	

Mean pD_2 values, intrinsic activities (α, (−)-isoprenaline = 1) and relative activities (RA, EC_{50} xamoterol:EC_{50} (−)-isoprenaline) are shown together with s.e.mean from *n* experiments.

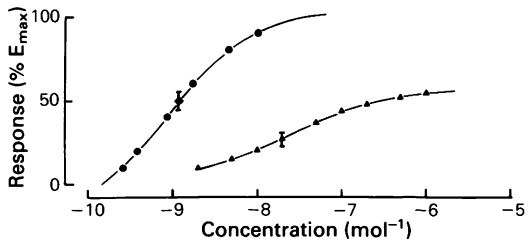

Figure 2 Mean concentration-effect curves for the ability of (−)-isoprenaline (●) and xamoterol (▲) to produce positive chronotropic actions in rat atrial preparations. Responses are expressed as a percentage of maximal response to (−)-isoprenaline. Error bars represent s.e.mean ($n = 4$) at half maximal effects for each drug.

to guinea-pig and rat atrial preparations does not appear to be a consequence of using non-reserpine-treated animals, since in two preparations from reserpinized cats (1 mg kg^{-1} i.p. 24 h), pD_2 values 7.88, 8.06, intrinsic activities 0.50, 0.54 and relative activities 7.64, 8.95 fell within the same range as those found in preparations from non-reserpine-treated animals.

Bolus doses of xamoterol (1–20 μmol l^{-1} in different experiments) produced chronotropic responses which were 52 and 59%, 13 and 31%, and 49 and 60% of the (−)-isoprenaline maximum in cat, guinea-pig and rat atrial preparations respectively. These fall within the same general range of responses obtained to equivalent doses of xamoterol reached through the cumulative administration of the compound in cats (12–50%), guinea-pigs (9–26%) and rats (39–75%). It would therefore appear that the cumulative dose scheme used does not introduce an undue bias into the expression of the agonistic responses to xamoterol.

Although (−)-isoprenaline was a potent agonist in driven guinea-pig left atrial and right ventricular strip preparations, xamoterol administered cumulatively (0.1 nmol l^{-1}–2 μmol l^{-1}) or by bolus dose (2 μmol l^{-1}) was devoid of positive inotropic effects in these preparations. In cat left atrial preparations, xamoterol possessed inotropic activity (Table 1), however, the responses were variable, relative activities ranging from 3 to 37 and intrinsic activities, from 0.04 to 0.50. Single bolus doses of the test compound (1 μmol l^{-1}) produced responses which fell within the range of maximal effects produced following its cumulative administration ($\alpha = 0.16$ and 0.20). The inotropic actions of xamoterol were reversed by atenolol (2 μmol l^{-1}) but were unaffected by ICI 118,551 (50 nmol l^{-1}).

In driven guinea-pig left atrial preparations in which the mean effective refractory period (ERP) under control conditions was 64 ms (s.e.mean = 3, $n = 9$), both (−)-isoprenaline and xamoterol (0.001–100 μmol l^{-1}) produced reductions in ERP. With both compounds there was a little or no change in the response over the concentration range 0.01–10 μmol l^{-1}, and when expressed as a percentage of control values (= 100%) the mean effective refractory periods obtained in the presence of 1 μmol l^{-1} of (−)-isoprenaline and xamoterol were 80% (s.e.mean = 6, $n = 4$) and 90% (s.e.mean = 2, $n = 4$) of their respective controls.

In separate experiments, the effects of (−)-isoprenaline and xamoterol (0.001–100 μmol l^{-1}) on ERP were monitored in the presence of β-adrenoceptor blockade with propranolol (1 μmol l^{-1}). In these experiments the mean control ERP was 96 ms (s.e.mean = 10, $n = 8$). While propranolol, as expected (Benfrey & Varma, 1967), prevented the decrease in ERP produced by (−)-isoprenaline, the effects of xamoterol were little changed or even enhanced. The mean ERP values obtained with 1 μmol l^{-1} (−)-isoprenaline and xamoterol in the presence of propranolol were 101% (s.e.mean = 8, $n = 4$) and 81% (s.e.mean = 4, $n = 4$) of their respective control values (= 100%).

Smooth muscle preparations

(−)-Isoprenaline produced concentration-related relaxant effects in carbachol-contracted guinea-pig tracheal and K^+-depolarized uterine preparations from guinea-pigs and rats, and inhibited electrically-induced contractions of the guinea-pig ileum and the rat vas deferens (see Table 1 for pD_2 values). All these effects of (−)-isoprenaline were antagonized by propranolol (0.5 μmol l^{-1}).

Xamoterol (0.1–50 μmol l^{-1}) produced a variety of effects in smooth muscle preparations (Table 1). The compound was generally without effect on the contractile activity of the rat vas deferens and had no effect on K^+-induced tone in oestrogen-pretreated rat uterine preparations, while in guinea-pig uterine preparations high concentrations of the compound (10–50 μmol l^{-1}) produced small, short-lasting increases in tension which returned to pre-exposure levels within 15 min. Bolus doses of xamoterol (20 μmol l^{-1}) were also without effects in these preparations.

In contrast, xamoterol produced a concentration-dependent inhibition of contractions in transmurally-stimulated guinea-pig ileal preparations (1–20 μmol l^{-1}), and relaxant effects in carbachol-stimulated guinea-pig tracheal (0.5 nmol l^{-1} –10 μmol l^{-1}) and progesterone-pretreated rat uterine (0.01–20 μmol l^{-1}) preparations.

Since the inhibitory effects of xamoterol were difficult to reverse on washing, the involvement of β-adrenoceptor-mediated actions in the responses was

initially tested by adding propranolol (0.5 μmol l^{-1}) to the bath in the continuing presence of xamoterol.

When expressed in terms of the maximal inhibitory effects produced by (−)-isoprenaline, xamoterol (10 μmol l^{-1}) produced a 29% (s.e.mean = 8, n = 6) relaxation in guinea-pig tracheal preparations and 20 μmol l^{-1} xamoterol a 69% (s.e.mean = 8, n = 4) inhibition of contractions in transmurally stimulated ileal preparations. These inhibitory effects were not reversed in the presence of propranolol.

In contrast, the relaxant effects produced by xamoterol (0.01–20 μmol l^{-1}) in progesterone-pretreated rat uterine preparations (range 24–48% of the maximal response to (−)-isoprenaline) were slowly reversed by propranolol and tone returned towards pre-xamoterol levels. This latter result suggested that in the uterus the inhibitory effect was mediated via β-adrenoceptor stimulation, a fact that was confirmed in three further experiments in which xamoterol, over the same concentration range used in the initial experiments, was without relaxant effects in the presence of propranolol (0.5 μmol l^{-1}).

In additional experiments, paired guinea-pig ileal and tracheal preparations taken from adjacent sections of the gut and trachea were set up and the actions of cumulatively administered xamoterol re-investigated in the presence and absence of β-adrenoceptor antagonists. In three paired ileal preparations, the inhibitory actions of the compound were similar in the absence and presence of propranolol (0.5 μmol l^{-1}, n = 2) or atenolol (1 μmol l^{-1}, n = 1). Although somewhat more variable, a similar lack of β-adrenoceptor mediated relaxation in tracheal preparations was indicated by the correspondence in the effects produced by xamoterol alone and in the presence of atenolol (1 μmol l^{-1}, n = 2), ICI 118,551 (0.1 μmol l^{-1}, n = 2) or propranolol (0.5 μmol l^{-1}, n = 1).

Table 2 Smooth muscle relaxant effects of xamoterol (20 μmol l^{-1}) in isolated tissues from the guinea-pig obtained in the presence of propranolol (1 μmol l^{-1})

Tissue	*Spasmogen*	*Tension*	*% inhibition*
Ileum	ACh	2.5 ± 0.1	28 ± 1
	Histamine	2.5 ± 0.1	69 ± 4
	Ca^{2+}	0.9 ± 0.4	68 ± 5
Trachea	ACh	0.6 ± 0.1	26 ± 3
	Ca^{2+}	0.4 ± 0.1	53 ± 4
Uterus	Ca^{2+}	0.6 ± 0.2	37 ± 7*

Mean tensions developed (g) by the various agonists are shown together with the % inhibition of responses by xamoterol; s.e.mean from 4 experiments are indicated. *In the uterus, the response to 0.1 μmol l^{-1} xamoterol is shown since this concentration produced maximal inhibition.

The nature of the non-β-adrenoceptor mediated effects of xamoterol (0.1–20 μmol l^{-1}) were investigated further in guinea-pig tracheal and ileal preparations in the continuing presence of propranolol (1 μmol l^{-1}). In these experiments xamoterol was added to the bath 2 min before challenge with a spasmogen.

In ileal preparations, contractions to concentrations of acetylcholine (0.1–0.2 μmol l^{-1}) or histamine (1–10 μmol l^{-1}) that produced submaximal responses (approximately 80% E_{max}) were first obtained, and thereafter responses were elicited in the presence of increasing concentrations of xamoterol. Xamoterol produced a concentration-related decrease in the contractions to both spasmogens. The mean inhibition of responses to acetylcholine and histamine produced with the highest dose of xamoterol used (20 μmol l^{-1}) were approximately 30 and 70% respectively (Table 2). Similarly, responses of tracheal preparations to acetylcholine (0.5–5 μmol l^{-1}) were also inhibited by xamoterol (Table 2).

In further experiments using ileal, tracheal and uterine preparations, the tissues were bathed in a calcium-free K^+-depolarizing solution (i.e. calcium-free Krebs solution in which all Na^+ salts were replaced with K^+ salts) and contractions were produced by the addition of calcium chloride to the bath. Xamoterol (in the presence of 0.5 μmol l^{-1} propranolol) produced a concentration-related decrease in responses to 10 mmol l^{-1} calcium in ileal and 5 mmol l^{-1} calcium in tracheal preparations (Table 2, Figure 3). In uterine preparations the maximal inhibition of responses to calcium (0.1–1 mmol l^{-1}) was obtained with 0.1 μmol l^{-1} xamoterol; at higher concentrations responses to calcium returned towards control levels (Table 2, Figure 3).

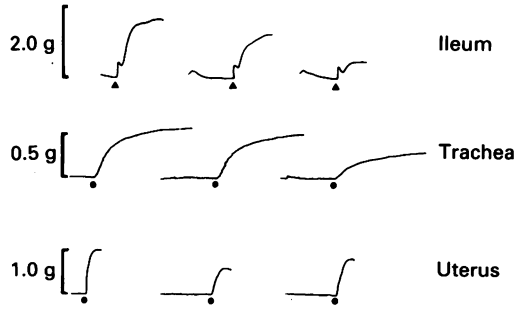

Figure 3 Traces of contractile responses to calcium in isolated smooth muscle preparations from the guinea-pig under control conditions (left panel) and in the presence of 0.1 μmol l^{-1} (centre panel) and 10 μmol l^{-1} xamoterol (right panel). Top panel shows responses to 0.1 mmol l^{-1} Ca^{2+} in ileal preparations, middle panel responses to 5 mmol l^{-1} Ca^{2+} in tracheal, and lower panel responses to 5 mmol l^{-1} Ca^{2+} in uterine preparations.

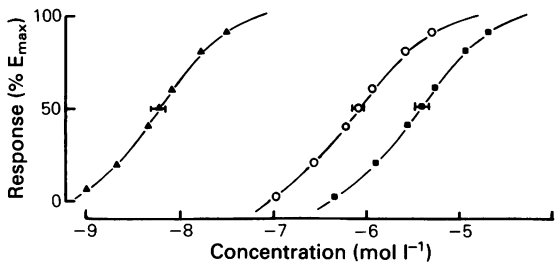

Figure 4 Mean concentration-effect curves ($n = 4$) for positive inotropic effects of (−)-isoprenaline in driven left atrial preparations from the guinea-pig in the absence (▲) and in the presence of xamoterol (O, 2 μmol l^{-1}; ■, 10 μmol l^{-1}). Responses are expressed as a percentage of the maximal responses to (−)-isoprenaline under control conditions. Horizontal bars show s.e.mean at the 50% E_{max} level.

Antagonistic actions at β-adrenoceptor sites

Concentration-effect curves for the positive chronotropic actions of (−)-isoprenaline in guinea-pig right atrial preparations, and for positive inotropic actions in driven guinea-pig and cat left atrial and guinea-pig ventricular preparations, were shifted to the right in a parallel concentration-dependent fashion by xamoterol. Maximal responses to (−)-isoprenaline were unaffected. Figure 4 shows mean concentration-effect curves to (−)-isoprenaline in the absence and presence of xamoterol in guinea-pig left atrial preparations. Analysis of the results in these cardiac preparations showed that the antagonism displayed with xamoterol was of a competitive type; pA_2 values ranged from 7.42 to 7.98 (Table 3).

Table 3 Antagonistic actions of xamoterol at β-adrenoceptor sites

Tissue	*Agonist*	*pA_2*	*Slope*	n
Guinea-pig				
Right atria	Isoprenaline	7.42 ± 0.03	0.91 ± 0.06	4
Left atria	Isoprenaline	7.80 ± 0.06	1.01 ± 0.11	4
Right ventricle	Isoprenaline	7.54 ± 0.06	1.28 ± 0.09	4
Ileum*	Isoprenaline	7.43 ± 0.16		4
Trachea	Isoprenaline	5.43 ± 0.09	0.90 ± 0.08	6
Trachea	Fenoterol	5.21 ± 0.12	0.94 ± 0.15	8
Trachea**	Noradrenaline	6.88 ± 0.04	1.10 ± 0.19	6
Cat				
Left atria	Isoprenaline	7.98 ± 0.07	1.00 ± 0.04	4
Rat				
Vas deferens	Isoprenaline	6.29 ± 0.10	1.18 ± 0.27	7

*pK_B value, see text for details
**Value obtained in the presence of 0.1 μmol l^{-1} ICI 118,551
Mean pA_2 values and slopes of the relationship between log (dose-ratio − 1) and log (xamoterol concentration) are shown together with s.e.means from n experiments.

The mean pK_B value for (−)-isoprenaline/xamoterol interaction in ileal preparations fell within the range of pA_2 values found in the cardiac preparations (Table 3). In these studies only one concentration of xamoterol (20 or 50 μmol l^{-1}) was used in each experiment, since the non-β-adrenoceptor mediated inhibitory response produced by the compound limited assessable shifts in superimposed concentration-effect curves to (−)-isoprenaline.

Xamoterol produced parallel rightward shifts in concentration-effect curves to (−)-isoprenaline, fenoterol and noradrenaline in guinea-pig tracheal preparations without affecting their maximal responses. As judged by the slope functions, only the interactions with (−)-isoprenaline and fenoterol were of a competitive type (Table 3). Slope values obtained with noradrenaline were significantly different from unity (mean 0.57, s.e.mean = 0.13, $n = 4$; 95% C.I., 0.15–0.98). However, when noradrenaline/xamoterol interactions were reassessed in the presence of the selective β_2-adrenoceptor antagonist ICI 118,551 (0.1 μmol l^{-1}) the slope was increased and was not significantly different from unity (Table 3).

In rat vas deferens preparations, xamoterol (10–75 μmol l^{-1}) produced parallel shifts in (−)-isoprenaline curves, and the slope function (mean = 1.18, s.e.mean = 0.27, $n = 7$) was not significantly different from unity. However, there was a very wide range of slope values in individual experiments (range 0.48–2.46), possibly related to the fact that at higher concentrations (> 20 μmol l^{-1}) xamoterol itself produced a variable depressant effect on the electrically-induced contractions of the preparation.

In guinea-pig uterine preparations, analysis of the dose-ratios obtained, for xamoterol-induced shifts in (−)-isoprenaline curves indicated that the antagonism was not of a competitive type since the mean slope value was significantly different from unity (mean slope = 1.19, s.e.mean = 0.02, $n = 4$; 95% C.I. 1.11–1.27).

Radioligand binding studies

Previous studies (McPherson *et al.*, 1984) have shown that the specific [^{125}I]-CYP binding sites on membrane homogenates of left atria and uterine tissues from guinea-pigs are saturable, of high affinity and possess the characteristics of homgeneous populations of β_1- and β_2-adrenoceptors respectively. Table 4 shows the dissociation constants (expressed as pK_D values) for the ability of xamoterol to displace the radioligand

Table 4 Dissociation constants (pK_D) and slope factors for displacement of [^{125}I]-iodocyanopindolol from guinea-pig left atrial and uterine membrane preparations

	n	pK_D	*Slope factor*
Left atria	4	7.25 ± 0.26	1.03 ± 0.09
Uterus	3	5.24 ± 0.03	1.17 ± 0.21

Mean values and s.e.mean from *n* experiments are shown

from β-adrenoceptor sites in the two tissues. The slope of the displacement curve was not different from unity in both tissues, indicating that displacement occurred from single sites in both tissues and analysis of the displacement data by LIGAND indicated a significant preference ($P < 0.05$) for one site rather than a two site model in each tissue.

Discussion

The results of the present experiments indicate that xamoterol can elicit β-adrenoceptor-mediated cardiac stimulant and smooth muscle relaxant effects in some, but not all isolated tissue preparations in which it was tested. Positive chronotropic effects were obtained in atrial preparations from cats, rats and guinea-pigs. In all cases, pD_2 values indicated that xamoterol had a high activity (relative activities with respect to (−)-isoprenaline ranged from 1–25), however, its intrinsic activity was low (< 0.55).

Even though cat (Broadley, 1982) and possibly guinea-pig (Johansson & Persson, 1983) right atrial preparations contain mixed populations of β_1- and β_2-adrenoceptors, it would appear that xamoterol produced its positive chronotropic actions through stimulation of β_1-adrenoceptors since its effects were reversed by the β_1-adrenoceptor selective antagonist atenolol but were unaffected by ICI 118,551, an antagonist with β_2-adrenoceptor selective actions.

Likewise, atenolol reversed the weak positive inotropic actions of xamoterol in cat left atrial preparations. The compound was without inotropic actions in driven left atrial and ventricular strip preparations from the guinea-pig. The variability in the cardiac responses to xamoterol in different species is reminiscent of results obtained with prenalterol (Kenakin & Beek, 1980; Mattsson *et al.*, 1982; Apperley *et al.*, 1982; Mattsson *et al.*, 1983). For prenalterol, Kenakin & Beek (1980) have suggested that the degree of agonistic activity obtained is related to the sensitivity of the preparations to isoprenaline, a feature which in turn gives some indication of the number of receptors available for activation and initiation of a response. While this idea might well be used to explain the lack of response to xamoterol in the ventricular preparations, in which (−)-isoprenaline has a low pD_2 value (7.50) it cannot be extended to guinea-pig left atria where the pD_2 value for (−)-isoprenaline (8.23) is similar to that found in right atria (8.30) in which xamoterol produces agonistic effects. Perhaps the suggestion by Mattsson *et al.* (1983) that tissue-dependent differences in the efficiencies of stimulus-response coupling may provide a better explanation for the observed phenomena.

In smooth muscle preparations, xamoterol produced clear β-adrenoceptor-mediated effects in only K^+-stimulated uterine preparations from progesterone pretreated rats, a tissue in which β_2-adrenoceptors are responsible for inhibitory activity, (Mattsson *et al.*, 1982; Mian, unpublished observation).

In the remaining smooth muscle preparations in which β_1-adrenoceptors (guinea-pig ileum; Mian *et al.*, 1984), β_2-adrenoceptors (oestrogen-pretreated guinea-pig uterus, rat vas deferens, oestrogen-pretreated rat uterus; Krstew *et al.*, 1982; Kenakin, 1982) or mixed β_1- and β_2-adrenoceptor populations (guinea-pig trachea; Iakovidis *et al.*, 1980; O'Donnell & Wanstall, 1981) subserve inhibitory responses, adrenoceptor-mediated actions of xamoterol were absent. However, xamoterol elicited non-β-adrenoceptor-mediated inhibitory actions in guinea-pig ileal and tracheal preparations.

These latter actions were confirmed in propranolol-treated preparations in which responses to both receptor agonists (acetylcholine and histamine) and to Ca^{2+} were depressed. These findings suggest that the inhibitory actions are non-specific and not related to interference with specific mechanisms by which tone was induced in the preparations. The ability of xamoterol to produce a propanolol-insensitive reduction in the effective refractory period of driven guinea-pig left atrial preparations is a further indicator of non-β-adrenoceptor mediated membrane action of the compound.

In organ bath studies, xamoterol produced a rightward shift of concentration-effect curves to (−)-isoprenaline and, with the exception of results obtained in guinea-pig and rat uterine preparations, the interactions were of a competitive type. Calculated pA_2 values for the antagonism of β-adrenoceptor-mediated effects in cardiac and in ileal preparations ranged from 7.42–7.98, values which are somewhat higher than the dissociation constant ($pK_D = 7.25$) of the drug for β_1-adrenoceptor binding sites in guinea-pig left atrial membrane preparations. In uterine membrane preparations (β_2-adrenoceptor binding sites), a pK_D of 5.24 was obtained, a value similar to the pA_2 values found for (−)-isoprenaline/xamoterol and fenoterol/xamoterol interactions in guinea-pig

tracheal preparations (5.43 and 5.21 respectively). In rat vas deferens preparations, in which a high degree of variability in slope values was noted, a mean pA_2 of 6.29 was obtained, whilst in uterine preparations from the guinea-pig and rat the non-competitive nature of the interactions precluded the calculation of valid pA_2 values.

It is tempting to speculate that non-β-adrenoceptor-mediated actions of xamoterol account for the variability in the pA_2 values for xamoterol at β_1- and β_2-adrenoceptor sites and hence the differences in the pA_2 and pK_D values obtained. The latter values are in accord with those of Cook *et al.* (1984) who published results of radioligand binding studies with xamoterol using rat and rabbit lung membrane homogenates.

In summary, the results of the present studies show that xamoterol has affinity for both β_1- and β_2-adrenoceptor sites. In this regard, pK_D values from binding studies indicate a 100 fold selectivity for the β_1-adrenoceptor subtype, a factor which is supported by xamoterol/agonist interactions as expressed by pA_2 values.

As an agonist, xamoterol shows tissue-dependent rather than receptor-dependent stimulant actions, therefore β_1-/β_2-adrenoceptor selectivity indices based on relative activities in different tissues are of little consequence in terms of their general predictive value.

The above properties of xamoterol suggest that it might best be compared with prenalterol in terms of its spectrum of pharmacological activity. With regard to affinity, xamoterol has a greater β_1-adrenoceptor selectivity than prenalterol, since pK_D values for the latter compound obtained from binding studies (Molenaar, personal communication) in guinea-pig left atrial (6.92) and in uterine homogenates (5.89) indicate only a 10 as opposed to the 100 fold selectivity shown with xamoterol. Cook *et al.* (1984) found a similar 10 fold selectivity for prenalterol using rat and rabbit lung homogenates. In terms of agonistic activity, comparisons of the actions of the two drugs indicate that in tissues where stimulant actions are present, the intrinsic activities displayed by prenalterol are greater than those of xamoterol, e.g. intrinsic activities for the positive chronotropic actions in atria from cats, rats and guinea-pigs are 0.87, 0.82 and 0.58 respectively for prenalterol (Mattsson *et al.*, 1983) and 0.31, 0.55 and 0.16 for xamoterol.

Although the non-specific smooth muscle relaxant effects of xamoterol are weak and only occur at higher concentrations than those required to produce cardiac stimulant effects, their possible presence may need to be taken into consideration when assessing the overall pharmacological activity of the compound.

In recent work Smith *et al.* (1984) have raised the possibility that the membrane stabilizing activity (MSA) that is produced with propranolol might involve a calcium blocking action. Whether or not this effect underlies the membrane-stabilizing actions common to many β-adrenoceptor antagonists is unknown. However, in view of the structural similarities between these compounds and xamoterol, and the Ca^{2+}/xamoterol interactions found in the present experiments, it is tempting to assign a 'calcium blocking' action to the smooth muscle relaxant effects of the latter compound. However, further work is required to confirm or reject this possibility.

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