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Comparison of the effects of xamoterol and isoprenaline on rat cardiac β-adrenoceptors: studies of function and regulation

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1 The effects of the β_1-selective partial agonist xamoterol and the full agonist isoprenaline on rat cardiac β-adrenoceptors were compared in functional studies of heart rate response *in vivo* and *in vitro*. In addition, the ability of both agents to cause receptor down-regulation in the rat heart following chronic (6 days) subcutaneous infusions was assessed by radioligand binding with [^{125}I]-pindolol.

2 In the functional studies, xamoterol produced a maximal effect equivalent to approximately 65% of that of isoprenaline and was overall less potent than the full agonist.

3 Compared to saline control, the density of β-adrenoceptors was reduced approximately 39% in ventricular membranes prepared from animals after 6 days of isoprenaline infusion but was unaffected by xamoterol. The relative proportions of the β-adrenoceptor subtypes were unchanged by either active treatment.

4 Plasma xamoterol level at the end of the infusion period was equivalent to that associated with maximum tachycardia *in vivo* and to the concentration producing maximal stimulation of the rat isolated atrium *in vitro*. Thus suggesting 100% β-adrenoceptor occupancy during the period of xamoterol infusion.

5 These results indicate that in this animal model xamoterol does not induce cardiac β-adrenoceptor down-regulation during chronic treatment, with doses that produce a maximal functional response both *in vitro* and *in vivo*.

Introduction

The β-adrenoceptor partial agonist xamoterol has recently been introduced into clinical practice for the treatment of patients with mild to moderate heart failure. In some animal models and normal healthy volunteers, it produces approximately 43% of the maximum response of the full agonist isoprenaline (Nuttall & Snow, 1982; Hashimoto *et al.*, 1986). The modest inotropic action of this drug is associated with a beneficial effect on stabilising sympathetic stimulation of the heart, providing inotropic support at low levels of sympathetic drive and protection from excessive and potentially metabolically harmful stimulation during exertion.

It is generally accepted that chronic agonist stimulation of β-adrenoceptors produces refractoriness (tachyphylaxis) and is usually associated with a reduction in tissue receptor density (down-regulation) and/or coupling to adenylate cyclase (Minneman *et al.*, 1981). Conversely chronic treatment with antagonists may induce receptor up-regulation (Aarons & Molinoff, 1982). Thus, attempts at chronic treatment of heart failure with the β-adrenoceptor agonist drug pirbuterol have been unsuccessful because of the rapid development of receptor desensitization and reduced responsiveness (Colucci *et al.*, 1984). Furthermore, in chronic heart failure β-adrenoceptor down-regulation has been demonstrated in hearts of transplant recipients, this is presumably related to the chronic sympathetic nervous stimulation characteristic of this condition (Bristow *et al.*, 1982; Bristow, 1984).

It is conceivable that β-adrenoceptor partial agonists may be of particular use in heart failure, being less likely to induce receptor down-regulation or tachyphylaxis of response during chronic treatment. Also they may, possibly, protect the cardiac β-adrenoceptors from 'adverse' regulatory influences of excessive noradrenergic discharge.

In view of the clinical interest in xamoterol, we have investigated the effects of this agent on the rat heart. Firstly, we established functional responsiveness compared to the full agonist isoprenaline and then determined the effect of chronic xamoterol treatment on cardiac muscle β-adrenoceptors.

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Methods

Functional studies

Two groups of experiments were performed.

Xamoterol-induced tachycardia in vivo *in anaesthetised rats* Female AP rats (300–350 g) were depeleted of catecholamine by dosing with syrosingopine ($5\,mg\,kg^{-1}\,day^{-1}$) for 2 days. Under pentobarbitone anaesthesia they were tracheotomised and bilaterally vagotomised. Cannulae were inserted for injection (Agla micrometer syringe) of isoprenaline (left jugular vein) and xamoterol (right jugular vein), measurement of blood pressure (left carotid artery) and collection of blood samples (right carotid artery). Heart rate was derived from the phasic blood pressure signal by a Nova HR2 heart rate meter.

Rats in groups of three were administered xamoterol by bolus injection in doses ranging from 0.1 to $33.0\,\mu g\,kg^{-1}$. Before xamoterol treatment, a dose-response curve to isoprenaline ($0.003–0.3\,\mu g\,kg^{-1}$) was established in each animal by cumulative dosing from the Agla micro syringe. Blood samples were collected following each isoprenaline dose-response curve (when heart rate had returned to baseline) and at the time of maximum increase of heart rate after xamoterol, which was approximately 1.5 min after bolus injection.

Increased heart rate in response to an injection of isoprenaline or xamoterol was expressed as a percentage of the maximum increase caused by injection of excess isoprenaline ($500\,\mu g\,kg^{-1}$) at the end of each experiment.

Xamoterol-induced tachycardia in rat isolated right atria Male AP rats (250–300 g) were killed by cervical dislocation and the hearts rapidly removed and placed in modified Krebs solution. The right atria were mounted between hooks in all glass, water-jacketed organ baths. The lower hook was anchored to the base of the bath and the upper to an isometric transducer (Dynometer, UF1). The resting tension of the preparation was adjusted to 0.5 g and the tissue maintained at 36°C in the modified Krebs buffer gassed with 95% O_2/5% CO_2. The rate of spontaneous beats was measured by linking the transducer signal to a cardiotachometer. A cumulative dose-response curve to isoprenaline (2×10^{-11} to 6×10^{-8} M)

was obtained first and then after washing the tissue. This was followed by a cumulative dose-response curve to xamoterol (2×10^{-10} to 6×10^{-6} M).

β-adrenoceptor regulation studies

Osmotic minipumps (Alza, Palo Alto, California, U.S.A.) loaded to administer either isoprenaline ($40\,\mu g\,kg^{-1}\,h^{-1}$; $n = 7$), xamoterol ($600\,\mu g\,kg^{-1}\,h^{-1}$; $n = 7$) or normal saline ($n = 9$) were implanted subcutaneously into AS rats (200–300 g) under halothane anaesthesia. All pumps were preincubated in normal saline at room temperature for 12 h before implantation to ensure rapid onset of infusion as per manufacturer's instructions. After 6 days animals were re-anaesthetised, blood samples for plasma isoprenaline or xamoterol measurement were taken by ventricular puncture and the hearts were carefully removed. The ventricles were dissected free of the atria and the membrane preparation was obtained from these fresh hearts. Binding studies were performed on the same day. Ventricular membranes were prepared by homogenisation and differential centrifugation by use of previously described methods (Baker *et al.*, 1980). Membranes were finally resuspended in assay buffer (50 mM Tris-HCl, 0.2 mM metabisulphate pH 7.8) at a final protein concentration of 0.5–1.0 mg ml^{-1}. Protein was determined by the method of Lowry *et al.* (1951).

[^{125}I]-(−)-pindolol was prepared with a theoretical specific activity of 2175 Ci mmol^{-1} according to the method of Barovsky & Brooker (1980) and was always used within 30 days. Membranes (100 μl) were incubated with the radioligand and appropriate concentrations of drugs in a final volume of 250 μl assay buffer for 40 min at room temperature, as previously described (Cook *et al.*, 1985). Specific binding to the ventricular β-adrenoceptors was defined as that displaced by 200 μM isoprenaline and was generally 80–90% of the total binding at the concentration of [^{125}I]-pindolol (50–60 pM) used in the competition experiments.

The relative proportions of the β-adrenoceptor subtypes in the tissue were assessed by performing saturation binding analysis of specific [^{125}I]-pindolol binding in the presence or absence of an appropriate concentration (3×10^{-7} M) of the highly selective β_1-adrenoceptor antagonist CGP 20712A (Dooley & Bittiger, 1984). The concentration of CGP 20712A required in these studies was confirmed, from graphical analysis of preliminary displacement experiments of [^{125}I]-pindolol binding to ventricular membranes, as that quantity of the selective compound which would occupy >95% of the β_1-adrenoceptors in this tissue (Figure 1 and Figure 2). The

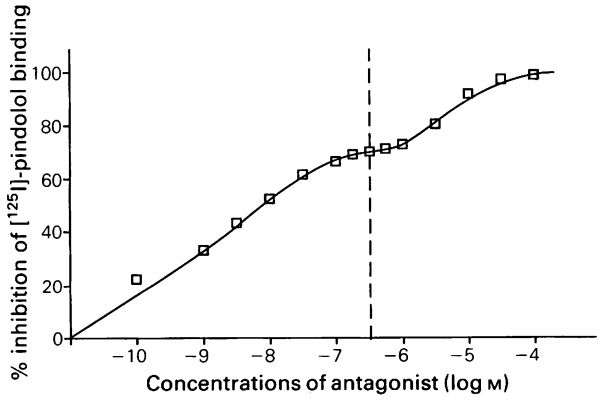

Figure 1 Displacement of [^{125}I]-pindolol binding to untreated rat ventricular membranes by CGP 20712A. Each data point represents the mean of 5–7 experiments (s.e.mean < ±10%). Broken line indicates displacement of [^{125}I]-pindolol binding by 3×10^{-7} M CGP 20712A and represents the occupation by this highly selective antagonist of >95% of the β_1-adrenoceptors in this tissue. The slope factor (nH) for CGP 20712A displacement curve is 0.1. Curve fitting analysis (Allfit) indicates a two site model with relative proportions of β_1- and β_2-adrenoceptors of 64% and 36%, respectively. The IC_{50} values of CGP 20712A for the subtypes are: β_1, 8.8×10^{-10} M and β_2, 2.6×10^{-5} M.

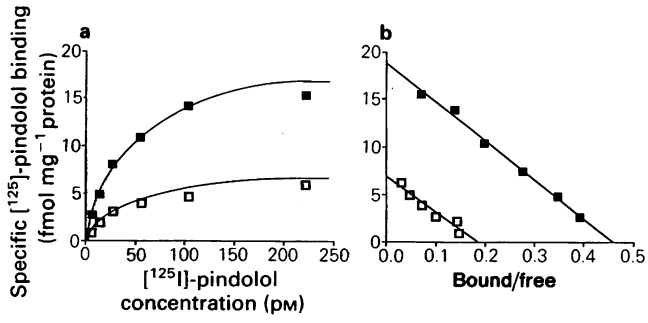

Figure 2 Saturation binding curves (a) and Scatchard analysis (b) of specific [^{125}I]-pindolol binding to rat ventricular membranes performed in the presence (□) and absence (■) of 3×10^{-7} M CGP 20712A. Data illustrated are from a single representative experiment. In the presence of CGP 20712A [^{125}I]-pindolol binds only to β_2-adrenoceptors. In the absence of CGP 20712A binding is to both subtypes (β_1 and β_2). B_{max} (fmol mg^{-1} protein) from the experiment illustrated: β_2, 6.5; β_1 (by subtraction from total binding), 10.7. The relative proportion of the subtypes (63% and 37%, β_1 and β_2, respectively) is in excellent agreement with that derived from curve fitting analysis of CGP 20712A displacement of [^{125}I]-pindolol binding (see Figure 1).

efficiency of β-adrenoceptor coupling to adenylate cyclase was assessed from the effects of the addition of 0.1 mM Gpp(NH)p (a non hydrolysable analogue of GTP) in competition binding experiments of isoprenaline displacement of [^{125}I]-pindolol binding in the presence of 8 mM $MgCl_2$.

Equilibrium dissociation constants (K_D) and binding site maxima (B_{max}) were determined by Scatchard analysis of saturation curves. IC_{50} values (concentrations producing 50% displacement of [^{125}I]-pindolol binding) and slope factors (nH) were determined from Hill plots. Plasma isoprenaline levels were measured by h.p.l.c. with electrochemical detection and xamoterol by radioimmunoassay.

Significant differences between mean values were assessed by Student's *t* test for unpaired means and all results are expressed as mean ± s.e.mean.

Materials

Na^{125}I (carrier free) was obtained from Amersham International, U.K., isoprenaline from Sigma Chemical Company and guanyl-5'-yl-imidophosphate (Gpp(NH)p) from Boehringer Mannheim. The following drugs were kindly donated as indicated: (−)-pindolol (Sandoz), xamoterol (ICI), CGP 20712A (2-hydroxy-5(2-((2-hydroxy-3-(4-((1-methyl-4-trifluoromethyl)-1H-imidazole-2-yl)-phenoxy)propyl)amino)-ethoxy)-benzamide monomethane sulphonate (Ciba-Geigy). Modified Krebs buffer contained (mM): NaCl 106, KCl 4.8, $MgSO_4$ 1.5, KH_2PO_4 1.2, $NaHCO_3$ 34, glucose 11.1, $CaCl_2$ 1.8, ascorbic acid 0.06 and dexamethasone 0.002.

Results

Functional studies

In both *in vivo* and *in vitro* experiments, xamoterol was shown to be a partial agonist producing a maximal effect equivalent to 65–70% of that of isoprenaline (Figures 3 and 4). *In vivo*, the maximal effect of xamoterol-induced tachycardia in the anaesthetised rats (1.5 min after bolus injection) was seen at a dose of $33\,\mu g\,kg^{-1}$ and a plasma level of 39.0 ± 5.0 ng ml^{-1} (115 nM). *In vitro*, the maximal increase of spontaneous atrial contraction was achieved with an organ bath concentration of xamoterol of approximately 140 nM. In addition, in both experiments xamoterol was approximately ten fold less potent than isoprenaline, as shown by comparison of EC_{50} and ED_{50} values (see legends Figures 1 and 2).

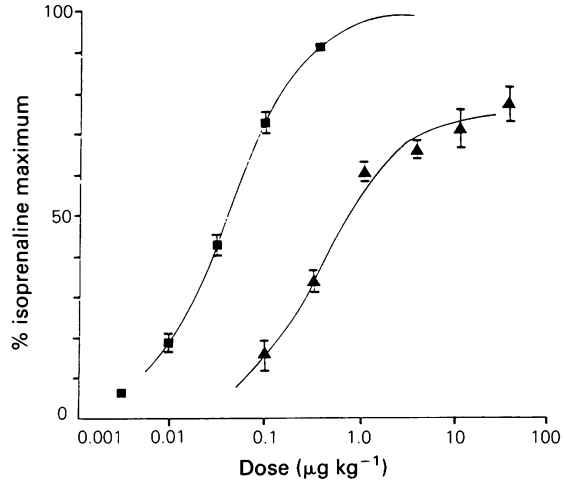

Figure 3 Isoprenaline (■) and xamoterol (▲) induced tachycardia in anaesthetised, vagotomised, catecholamine-depleted rats (computer predicted best fit dose-response curves). Each point represents the mean of experiments in three separate animals; vertical lines show s.e.mean. Mean ED_{50} values ($\mu g\,kg^{-1}$) were 0.04 and 0.361 for isoprenaline and xamoterol, respectively.

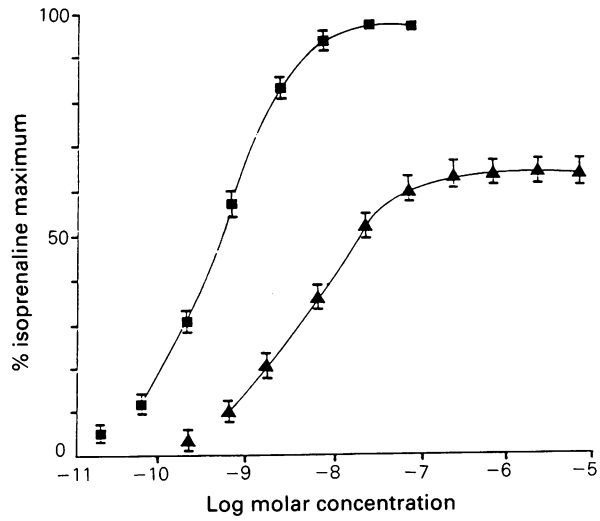

Figure 4 Effect of isoprenaline (■) and xamoterol (▲) on the rat isolated right atrium (for experimental details see text). Each point represents mean ($n = 11$) and vertical lines show s.e.mean. Computer predicted best fit dose-response curves. Mean EC_{50} values (nM) were 0.45 and 4.67 for isoprenaline and xamoterol, respectively.

Binding studies

Specific binding of [^{125}I]-pindolol to rat ventricular membranes was saturable and of high affinity (Figure 5). The displacement of [^{125}I]-pindolol binding by adrenoceptor agonists and antagonists was characteristic of a β-adrenoceptor, as has been demonstrated in other tissues (Cook *et al.*, 1985). The results of the saturation analysis performed in the presence of CGP-20712A indicated a heterogeneous population of β-adrenoceptor subtypes, with the proportion of β_1-adrenoceptors in the control membranes being 69 ± 3%. Compared to saline-treated animals, the maximal binding capacity of [^{125}I]-pindolol was reduced by approximately 39% after isoprenaline infusion but was unaffected by xamoterol (Table 1). The proportion of β_1-adrenoceptors was not significantly altered by either treatment, although a trend towards a greater degree of down-regulation of β_2-adrenoceptors was noted after isoprenaline (Table 1). Equilibrium dissociation constants were unchanged by any treatment (Figure 5 and legend). Gpp(NH)p produced a characteristic rightward shift (approximately three fold) and steepening of the isoprenaline displacement curve in control membranes. This effect and the associated overall change in the IC_{50} of isoprenaline in the presence or absence of the guanine nucleotide was not significantly different after any treatment (Table 2).

At the end of the study, plasma xamoterol and isoprenaline levels were 161 ± 21 nM and 7.3 ± 1.2 nM, respectively.

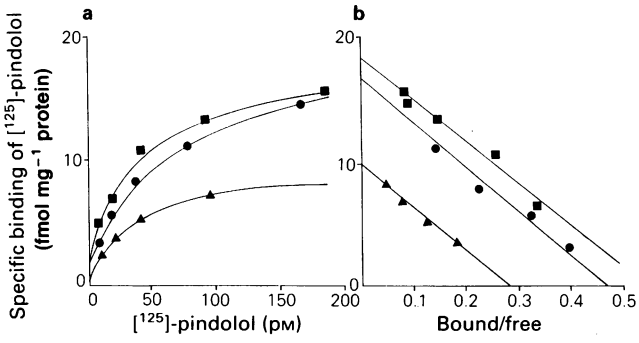

Figure 5 Specific [^{125}I]-(−)-pindolol binding to rat ventricular membranes prepared from animals chronically infused with saline (■), isoprenaline (▲) and xamoterol (●). Saturation data (a) and Scatchard analysis (b) are shown from single representative experiments. Mean B_{max} values are shown in Table 1. Mean K_D values (pM) were 35 ± 2 (saline), 42 ± 3 (isoprenaline) and 38 ± 2 (xamoterol). Numbers of experiments as indicated in the Methods section.

Discussion

The results of these studies indicate that in the rat heart the agonist activity of xamoterol on heart reate is approximately 65–70% of that of the full agonist isoprenaline. This is in contrast to data from other animal models (e.g. the dog) where

Table 1 Density of β-adrenoceptor subtypes (B_{max}) in rat ventricular membranes after 6 days subcutaneous infusions of saline, isoprenaline and xamoterol

	Control (saline)	*Isoprenaline*		*Xamoterol*	
β-adrenoceptors	B_{max}	B_{max}	*% change**	B_{max}	*% change*
Total	17.1 ± 1.1	10.5 ± 0.5**	−38.5	17.2 ± 1.4	0
β_1	11.2 ± 1.9	7.9 ± 0.7**	−29.5	11.8 ± 1.2	0
β_2	4.9 ± 0.8	2.9 ± 0.4**	−40.8	5.3 ± 0.6	+8.0

The values for B_{max} are mean ± s.e.mean (fmol mg^{-1} protein).
* change of mean B_{max} from control value.
** Indicates significantly different from control value $P < 0.05$; number of experiments as indicated in the text.

Table 2 Overall IC_{50} values and Hill slope factors (nH) for (−)-isoprenaline competition curves with [^{125}I]-pindolol binding to rat ventricular membranes after various treatments

Treatment	IC_{50} (nH)* No addition	With 0.1 mM Gpp(NH)p
Saline ($n = 9$)	$5.9 \pm 0.6 \times 10^{-8}$ (0.74)	$1.5 \pm 0.1 \times 10^{-7}$** (0.80)
Isoprenaline ($n = 7$)	$4.6 \pm 0.4 \times 10^{-8}$ (0.74)	$1.2 \pm 1.4 \times 10^{-7}$** (0.81)
Xamoterol ($n = 7$)	$9.1 \pm 1.7 \times 10^{-8}$ (0.77)	$2.7 \pm 0.34 \times 10^{-7}$** (0.92)

* IC_{50} values shown are mean ± s.e.mean (M) from the number of separate experiments shown. Hill slopes are calculated by graphical analysis from the means of the competition curves. All experiments were performed in the presence of 8 mM $MgCl_2$.
** Indicates values significantly different from that in the absence of Gpp(NH)p ($P < 0.05$).

the partial agonist activity of xamoterol has been formed to be approximately 43% of that of isoprenaline (Nuttall & Snow, 1982).

Chronic treatment with xamoterol in this model did not induce down-regulation of ventricular β-adrenoceptors when compared to isoprenaline. In addition, the proportion of β-adrenoceptors in the tissue was unchanged after xamoterol treatment. This suggests that xamoterol, as a β_1-selective agonist, did not cause down-regulation of β_1-adrenoceptors associated with a 'compensatory' increase in β_2-adrenoceptors, thus, it did not change the total receptor density.

Gpp(NH)p produced an identical and characteristic decrease in agonist affinity and steepening of the agonist binding isotherms in both control and treated ventricular membranes. This implies effective coupling between the β-adrenoceptor and adenylate cyclase, indicative of the formation of a complex between agonist, receptor and guanine nucleotide binding protein. Thus, xamoterol treatment did not appear to modify this coupling. In addition, the β-adrenoceptor population remaining after chronic isoprenaline treatment also exhibited unchanged agonist interactions.

There is a proportional relationship between receptor occupancy and functional response for partial agonists and maximal response requires 100% receptor occupancy. This is not so for full agonists, where, dependent on tissue and species, a variable proportion of 'spare' receptors may exist in association with maximal functional response. This is well demonstrated in the results from the present study of the effects of isoprenaline on the rat heart. The EC_{50} for isoprenaline stimulation of the rat isolated atria (0.45 nM, Figure 3) was approximately 150 fold lower than that demonstrated from isoprenaline displacement of [^{125}I]-pindolol binding to ventricular membranes (IC_{50} 70 nM, data not shown). In addition, maximal functional response to isoprenaline was observed *in vitro* at an organ bath concentration of approximately 10 nM, this is similar to the plasma concentration of isoprenaline measured at the end of the 6 day infusions *in vivo* (7 nM) and is equivalent to a receptor occupancy of approximately 10–15%. The plasma concentration of xamoterol (160 nM) at the end of the 6 day infusion was similar to that found associated with maximal tachycardia *in vivo* (115 nM) and to the organ bath concentration producing maximal stimulation of the rat isolated atrium (140 nM). This indicates strongly that during the period of xamoterol infusion *in vivo*, 100% receptor occupancy was achieved. Thus, during the infusions, xamoterol plasma concentrations maintaining full functional response and receptor occupancy were achieved without an alteration in receptor density.

No isoprenaline was detected in ventricular membranes from treated animals. In addition, neither total [^{125}I]-pindolol binding to the membranes nor β-adrenoceptor B_{max}, assessed by saturation analysis, was altered by addition of Gpp(NH)p after any treatment (data not shown). Thus, it is unlikely that 'apparent' down-regulation was produced by the retention of agonist tightly bound in the membranes, as has been suggested in other similar experimental situations (Nerme *et al.*, 1985).

In conclusion, therefore, we have demonstrated that the β_1-adrenoceptor partial agonist xamoterol exhibits 65–70% of the maximal functional activity of isoprenaline in the rat heart. In addition, in this animal model chronic treatment with xamoterol does not induce receptor down-regulation. This theoretically predictable result has important implications for the use of this drug in the long-term therapy of chronic heart failure.

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