British Journal of Pharmacology (2004) 142, 51–56
www.nature.com/bjp

Markedly reduced effects of (−)-isoprenaline but not of (−)-CGP12177 and unchanged affinity of β-blockers at Gly389-β_1-adrenoceptors compared to Arg389-β_1-adrenoceptors

[1]S.S. Joseph, [1]J.A. Lynham, [2]A.A. Grace, [1]W.H. Colledge & *,[1]A.J. Kaumann

[1]Department of Physiology, University of Cambridge, Downing Street, Cambridge CB2 3EG and [2]Papworth Hospital, NHS Trust, Papworth Everard, Cambridge, CB3 8ARE

1 Substitution of arginine by glycine at position 389, a frequent β_1-adrenoceptor polymorphism, reduces adenylyl cyclase stimulation by (−)-isoprenaline. β_1-Adrenoceptors mediate the effects of catecholamines and nonconventional partial agonists ((−)-CGP12177) through different sites. We investigated the influence of the 389 polymorphism on β blocker affinity, as well as on the responses to (−)-isoprenaline and the nonconventional partial agonist (−)-CGP12177 on cyclic AMP levels in CHO cells expressing recombinant Arg389-β_1-adrenoceptors (101 fmol mg^{-1} protein) or Gly389-β_1-adrenoceptors (94 fmol mg^{-1}).

2 The affinity of β-blockers and partial agonists, estimated from competition binding with (−)-[^{125}I]-cyanopindolol, was not different for Arg389-β_1-adrenoceptors and Gly389-β_1-adrenoceptors.

3 The maximum cAMP increases by (−)-isoprenaline and (−)-CGP12177 at Gly389-β_1-adrenoceptors were reduced by 97 and 46%, but the potencies enhanced 2 and 0.5 log units, respectively, compared to Arg389-β_1-adrenoceptors. The intrinsic activity of (−)-CGP12177 with respect to the (−)-isoprenaline was 0.057 at Arg389-β_1-adrenoceptors and 1.05 at Gly389-β_1-adrenoceptors.

4 We confirm in intact CHO cells that responses to (−)-isoprenaline are markedly reduced at Gly389-β_1-adrenoceptors compared to Arg389-β_1-adrenoceptors. However, the 389 polymorphism reduces considerably less the agonist responses to (−)-CGP12177, indicating that coupling to G_s protein is different for β_1-adrenoceptors activated by catecholamines than for receptors activated by nonconventional partial agonists. The affinity of β-blockers is conserved across the Arg389Gly polymorphism.

British Journal of Pharmacology (2004) **142,** 51–56. doi:10.1038/sj.bjp.0705753

Keywords: Recombinant Arg389- and Gly389-β_1-adrenoceptors; β-blockers; (−)-isoprenaline; (−)-CGP12177

Abbreviations: (−)-CGP12177, [(−)-4-3-(tertiarybutylamino-2-hydroxypropoxy) benzimidazol-2-one-hydrochloride]; (−)-RO363, ((−)-1-(3,4-dimethoxy-phenylethylamino)-3-(3,4-dihidroxy)-2-propanol)oxalate

Introduction

Human β_1-adrenoceptors present the common polymorphism Arg389Gly (Maqbool *et al*., 1999; Mason *et al*., 1999; reviewed by Small *et al*., 2003). Arg389-β_1-adrenoceptors and Gly389-β_1-adrenoceptors, expressed into Chinese hamster fibroblasts (CHF cells), mediate isoprenaline-evoked stimulation of adenylyl cyclase, but the maximum response in the Gly389 variant is only 1/3 of that of the Arg389 variant (Mason *et al*., 1999). Residue 389 is in the G_s-coupling domain of the β_1-adrenoceptor and Gly is believed to disrupt the predicted α-helix in this region, thereby reducing coupling to G_s protein (Small *et al*., 2003). Evidence obtained from agonist binding and [^{35}S]-GTPγ-S is consistent with reduced G_s-coupling of Gly389 receptors compared to Arg389 receptors (Mason *et al*., 1999).

There is controversy about the clinical significance of the Arg389Gly polymorphism. Exercise-induced increase in heart rate appeared to be unaffected by the Arg389Gly polymorphism (Buscher *et al*., 2001; Xie *et al*., 2001) in healthy volunteers. In contrast, heart failure patients homozygous for the Gly389 polymorphism had depressed exercise performance compared to those with Arg389-β_1-adrenoceptors (Wagoner *et al*., 2002). The positive inotropic effects of noradrenaline on robust trabeculae from human atria, usually from nonfailing hearts of patients with coronary heart disease, were not affected by the Arg389Gly polymorphism (Molenaar *et al*., 2002), but were enhanced in weak trabeculae from Arg389 patients compared to weak atrial trabeculae from Gly389 patients (Sandilands *et al*., 2003). There appears to be no association with the 389 polymorphism on the blood pressure or heart rate responses to β-blockers in the treatment of hypertension (O'Shaughnessy *et al*., 2000). In contrast, studying the effects of atenolol and metoprolol on healthy volunteers, Liu *et al*. (2003) and Sofowora *et al*. (2003), respectively, found that Arg389 homozygous subjects showed larger decreases in systolic blood pressure and mean blood pressure than Gly389 homozygous subjects. In addition, Liu *et al*. (2003) also reported greater metoprolol-evoked bradycardia in homozygous Arg389 than homozygous Gly389. Furthermore, homozygous Arg389 patients with end-stage

*Author for correspondence; E-mail: ajk41@hermes.cam.ac.uk
Advance online publication: 22 March 2004

heart failure, but not homozygous Gly389 patients, showed significant improvement of the left ventricular ejection fraction when treated with the β-blocker carvedilol (Perez *et al*., 2003). The more marked effect of atenolol and metoprolol, as well as the selective effect of carvedilol, could be due to higher affinity of these β-blockers for Arg389-β_1-adrenoceptors than Gly389-β_1-adrenoceptors. We therefore examined the affinity of atenolol and carvedilol, as well as the affinity of other β-blockers, for the two recombinant variants.

β_1-Adrenoceptors mediate the cardiac effects of catecholamines that are antagonized with high affinity by β-blockers. β_1-Adrenoceptors also mediate the cardiac effects of nonconventional partial agonists (NCPA) (e.g. (−)-CGP12177) that are partially resistant to β-blockers in mammalian heart (Kaumann, 1989; Lowe *et al*., 2002), including man (Kaumann, 1996; Joseph *et al*., 2003; Sarsero *et al*., 2003). Two binding sites for (−)-(^{3}H)-CGP12177) have been detected in rat left atrium (Sarsero *et al*., 1999) and human right atrium (Sarsero *et al*., 2003). Through the high-affinity site β-blockers, including (−)-CGP12177, antagonize the effects of catecholamine. The low-affinity site usually appears to mediate the effects of NCPA (but see Baker *et al*., 2003), including those of (−)-CGP12177, and has low affinity for β-blockers (Kaumann, 2000; Konkar *et al*., 2000; Granneman, 2001; Lowe *et al*., 2002; Joseph *et al*., 2003). The two binding sites for (−)-[^{3}H]-CGP12177 (affinity ratio $\sim$500) were recently verified on recombinant Arg389-β_1-adrenoceptors transfected at physiological density into CHO cells (Joseph *et al*., 2004).

(−)-CGP12177 enhances cAMP levels and stimulates cAMP-dependent protein kinase activity in the atria of rat (Kaumann and Lynham, 1997; Kaumann *et al*., 1997) and man (Sarsero *et al*., 2003) through β_1-adrenoceptors. Although this is consistent with stimulation of each of the two β_1-adrenoceptor sites leading to G_s protein, it does not necessarily imply that coupling is through the same receptor constituents for (−)-isoprenaline and (−)-CGP12177. The decreased β_1-adrenoceptor coupling to G_s of the Gly389 variant, compared to the Arg389 variant (Mason *et al*., 1999), was described for recombinant receptors activated by isoprenaline. To investigate whether or not the Arg389Gly polymorphism affects the responses to a catecholamine and an NCPA differently, we compared the cAMP-enhancing effects of (−)-isoprenaline and (−)-CGP12177 in CHO cells, expressing either the Gly389 variant or Arg389 variant. To examine whether the binding of β-blockers to β_1-adrenoceptors depends on the Arg389Gly polymorphism, we compared the affinities of β-blockers for the Gly389 variant and Arg389 variant, labelled with (−)-[^{125}I]-cyanopindolol. The catecholamine responses, but not the (−)-CGP12177 responses, were markedly reduced in the Gly389 variant compared to the Arg389 variant. The affinity of β-blockers did not differ between the variants.

Methods

Generation of Arg389 and Gly389 β_1-adrenoceptors expressed in CHO cells

A 2.4 kb full-length cDNA of the human β_1-adrenoceptor, cloned into the *Eco*RI/*Kpn*I site of the vector pcDNA3, was obtained as a gift from Dr Graeme Milligan (University of Glasgow, U.K.). When sequenced, the insert was found to consist of the arginine389 polymorphic variant (Mason *et al*., 1999). The Arg389-β_1-adrenoceptor cDNA was cloned into the pBI-L plasmid (Clontech), which also contains a luciferase reporter gene regulated from a tetracycline response element to allow surrogate monitoring of β_1-adrenoceptor gene expression. The pBI-L Arg389 β_1-adrenoceptor plasmid was co-transfected with a pTK-Hyg vector into the CHO-AA8 cell line (Gossen & Bujard, 1992) and stable hygromycin-resistant cell lines were selected. Nine out of 35 hygromycin-resistant clones showed high levels of luciferase activity and these nine clones were analysed for β_1-adrenoceptor density using a binding assay with (−)-[^{125}I]-cyanopindolol (Lowe *et al*., 2002). A clone with a β_1-adrenoceptor density of 101 ± 8.6 fmol mg^{-1} protein ($K_D = 13.4 \pm 1.1$ pM, $n = 13$ assays in duplicate) was used for cAMP assays.

A Gly389-β_1-adrenoceptor cDNA clone was generated as follows: PCR primers were used to amplify the region of the β_1-adrenoceptor gene surrounding the 389 amino-acid codon (forward primer 5′ TCGCCATCACCTCGCCCTTCCGCTACCAGA 3′, reverse primer 5′ CGCCGGGCCCTACACCTTGGA 3′) in genomic DNA from six human right atrial heart tissue samples. The PCR products were digested with *Bsm*FI to determine which variant they corresponded to, and one product showed that it contained the Gly389-β_1-adrenoceptor variant (*Bsm* FI digestion only occurs at residue 389 if glycine is present). This product was then digested with *Xho*I and *Asc*I, and subcloned into a recombinant β_1-adrenoceptor pBI-L vector, from which the corresponding Arg389 variant fragment of the cDNA had been removed. The resulting Gly389-β_1-adrenoceptor pBI-L plasmid was then sequence-verified before co-transfection with pTK-Hyg as described above, to obtain a stable cell line. After selection in hygromycin for 3 weeks, a stably expressing Gly389 β_1-adrenoceptor clone was obtained, by confirming expression of the luciferase gene and by determination of β_1-adrenoceptor density using (−)-[^{125}I]-cyanopindolol. A clone with a β_1-adrenoceptor density of 93.5 ± 19.6 fmol mg^{-1} protein ($K_D = 11.7 \pm 1.3$ pM, $n = 8$ assays in duplicate) was used for cAMP assays.

Receptor-binding assays

CHO-AA8 cells with stably transfected β_1-adrenoceptors, stored at −80°C in 1 mM EDTA, 25 mM Tris–HCl, pH 7.4 (20°C) were thawed and pelleted for 30 min at 17,000 × *g*, 4°C, and then homogenized by Polytron (7 mm probe, 3 × 10 s, speed setting 8) in a binding buffer containing (mM): EGTA 5, EDTA 1, $MgCl_2$ 4, ascorbic acid 1, phenylmethyl-sulfonyl fluoride 0.5, Tris–HCl 50, pH 7.5 (37°C). For receptor saturation assays, homogenates were incubated for 2 h at 37°C in a final volume of 0.5 ml (10 μg protein) with 1–200 pM (−)-[^{125}I]cyanopindolol. Nonspecific binding was defined as binding not removed by 200 μM (−)-isoprenaline. Specifc binding was not detected for (−)-[^{125}I]-cyanopindolol in cells that did not express β_1-adrenoceptors. The bound radioligand was isolated by filtration through Whatman GF/B paper and radioactivity counted in a gamma scintillation spectrometer. To estimate the affinity of β-blockers for the β_1-adrenoceptor, cell membranes were labelled with $\sim$30 pM (−)-[^{125}I]-cyanopindolol in the absence and presence of concentrations (0.03 nM–100 μM, spaced by 0.5 log units) of the competing

ligand. Binding assays were carried out in duplicate and replicated thrice.

Cyclic AMP assay

CHO-AA8 cells (Clontech) were grown to 70% confluence in 24-well plates containing Minimum Essential Medium (alpha modification) and 100 μg ml^{-1} each of G418 and hygromycin. Cells were washed with warm (37°C) phosphate-buffered saline and pre-incubated for 20 min at 37°C with Dulbecco's modified Eagle's medium buffered with 25 mM HEPES, containing 1 mM IBMX and 0.2 mM ascorbic acid in the absence or presence of (−)-propranolol. Cells ($\sim 10^5$ per well) were incubated with the indicated concentrations of agonists or forskolin (100 μM) for 20 min at 37°C, followed by aspiration of the medium and immediate addition of $HClO_4$ (0.3 M). Cell extracts were neutralized with 1.25 volumes of a 50/50(v v^{-1}) mixture of tri-*n*-octylamine/1,1,2-trichlorotrifluoroethane and the aqueous supernatant assayed with Amersham's Biotrak cAMP double antibody kit. Assays were carried out in triplicate and replicated three to five times.

Protein was determined by the bicinchoninic acid method against a bovine serum albumin standard.

Statistics

Data from saturation binding and inhibition of binding by competing ligands were analysed by nonlinear regression using GRAFIT3 (Leatherbarrow, 1992). Data are presented as mean ± s.e.m. Student's *t*-tests were used to test the differences between two groups of data.

Drugs

(−)-CGP12177 and carvedilol were gifts of GlaxoSmithKline (Harlow, Essex, U.K.). (−)-Bupranolol was a gift of Schwarz Pharmaka (Monheim, Rheinland, Germany). Oxprenolol was a gift of Novartis (Basle, Switzerland). Xamoterol was a gift of Zeneca (Macclesfield, U.K.). (−)-Isoprenaline hydrochloride, (−)-alprenolol, (−)-atenolol, metropolol, nadolol, (−)-pindolol, (−)-propranolol and (−)-timolol were purchased from Sigma (Poole, U.K.). Bisoprolol and sotalol were purchased from Tocris (Bristol, U.K.). (−)-Denopamine was from Tanabe Seiyaku (Osaka, Japan). (−)-RO363 was from the Institute of Drug Technology (Boronia, Australia).

Results

Binding of β-blockers to Arg389 and Gly389-β_1-adrenoceptors

The β-blockers competed with (−)-[^{125}I]-cyanopindolol for binding to β_1-adrenoceptors. The dissociation equilibrium constants K_i, calculated using K_D values of 13.4 and 11.7 pM for (−)-[^{125}I]-cyanopindolol on Arg389-β_1-adrenoceptors and Gly389-β_1-adrenoceptors, respectively, are listed in Table 1. The affinities of β-blockers, including partial agonists, did not differ between the Arg389 and Gly389 variants (Table 1, Figure 1).

Table 1 Comparison of binding constants (K_i, nM) of β-blockers and partial agonists for Arg389-β_1-adrenoceptors and Gly389-β_1-adrenoceptors

	Arg389-β-*adrenoceptor*	*Gly389*-β-*adrenoceptor*
(−)-[^{125}I]-cyanopindolol	0.0134 ± 0.0011	0.0117 ± 0.0013
(−)-CGP12177	0.36 ± 0.05	0.58 ± 0.11
(±)-Carvedilol	1.1 ± 0.1	1.7 ± 0.2
(−)-Pindolol	2.8 ± 0.1	3.0 ± 0.4
(±)-Oxprenolol	3.9 ± 0.3	4.2 ± 0.4
(±)-Timolol	4.4 ± 0.3	4.3 ± 0.5
(−)-Bupranolol	5.1 ± 0.41	6.0 ± 0.8
(−)-Propranolol	5.6 ± 0.7	6.5 ± 0.9
(−)-Alprenolol	7.9 ± 0.3	8.9 ± 0.9
(±)-Nadolol	22 ± 5	22 ± 2
(−)-RO363	30 ± 8	41 ± 4
(±)-Bisoprolol	97 ± 7	99 ± 20
(±)-Metoprolol	173 ± 11	156 ± 35
(±)-Xamoterol	169 ± 14	227 ± 19
(−)-Atenolol	865 ± 85	1372 ± 258
(−)-Denopamine	1601 ± 18	2350 ± 318
(±)-Sotalol	4611 ± 374	4577 ± 417

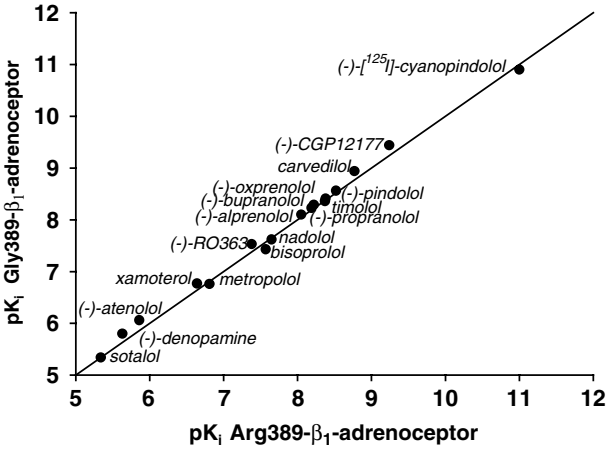

Figure 1 Comparison of binding affinities of β-blockers and partial agonists for Arg389-β_1-adrenoceptors and Gly389-β_1-adrenoceptors labelled with (−)-[^{125}I]-cyanopindolol (data from Table 1).

Increases of cAMP by (−)-isoprenaline and (−)-CGP12177 through Arg389- and Gly389-β_1-adrenoceptors

The maximum effects of (−)-isoprenaline were markedly reduced at the Gly389 variant to only 3% of the E_{max} for the Arg389 variant, but the potency was 98 times greater for the Gly389 variant than the Arg389 variant (Figures 2 and 3, Table 2).

(−)-CGP12177 was a weak partial agonist with intrinsic activity 0.057 with respect to (−)-isoprenaline on Arg389-β_1-adrenoceptors (Figures 2 and 3, Table 2). On the Gly389 variant, (−)-CGP12177 was a full agonist (intrinsic activity 1.05 with respect to (−)-isoprenaline), with a three-fold greater potency than on the Arg389 variant (Figures 2 and 3, Table 2). E_{max} of (−)-CGP12177 from the Gly389 variant was 54% of the E_{max} from the Arg389 variant (Table 2).

(−)-Propranolol (200 nM) caused a 2 log unit shift of the concentration–effect curves of (−)-isoprenaline, but only

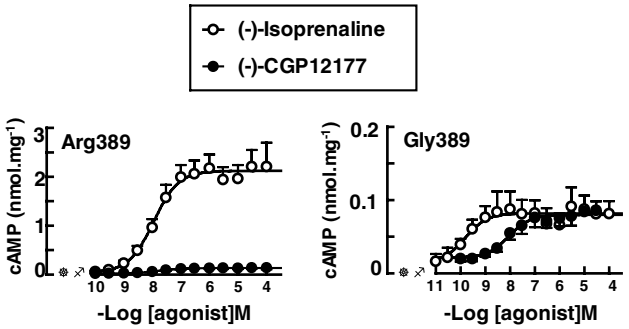

Figure 2 Comparison of the effects of (−)-isoprenaline and (−)-CGP12177 on cAMP levels of CHO cells expressing Arg389-β_1-adrenoceptors and Gly389-β_1-adrenoceptors. For further details, see Table 2.

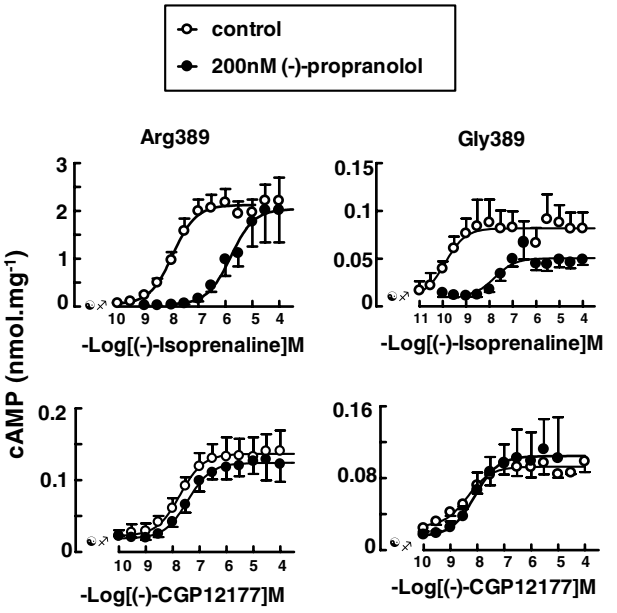

Figure 3 Antagonism by (−)-propranolol of the effects of (−)-isoprenaline and (−)-CGP12177 at Arg389-β_1-adrenoceptors and Gly389-β_1-adrenoceptors. For further details, see Table 2.

marginal antagonism of the effects of (−)-CGP12177 in both Arg389 and Gly389 variants (Figure 3, Table 2). The high- and low-affinity estimates (pK_B values) of (−)-propranolol for the sites activated by (−)-isoprenaline and (−)-CGP12177, respectively, were similar for both variants (Table 2).

Forskolin (100 μM) increased cAMP by 3012 ± 361 (from 26.0 ± 3.6) and 2921 ± 585 (from 24.1 ± 3.7) pmol mg^{-1} in CHO cells expressing the Arg389 ($n = 11$) and Gly389 ($n = 12$) variant, respectively, demonstrating that the transfection of the variants did not change the catalytic activity of the adenylyl cyclase.

Discussion and conclusions

We found that the affinity of β-blockers and the partial agonists (−)-RO363 (Molenaar *et al*., 1997), (−)-denopamine (Yabana *et al*., 1992), xamoterol (Lemoine *et al*., 1989) and (−)-CGP12177 is essentially the same for the site labelled by (−)-[^{125}I]-cyanopindolol at Arg389-β_1-adrenoceptors and Gly389-β_1-adrenoceptors. We confirm the findings of Mason *et al*. (1999) that the agonist effects of (−)-isoprenaline are markedly reduced in the Gly389 variant compared to the Arg389 variant. However, the Arg389Gly polymorphism affects considerably less the agonist effects (−)-CGP12177 than those of (−)-isoprenaline. Gly389 appears to disrupt the formation of an α-helicoid, which is formed with the Arg389, thereby reducing coupling to G_s protein (Small *et al*., 2003). Our results suggest that this mechanism seems to reduce considerably more the effects of (−)-isoprenaline than the effects of (−)-CGP12177. Thus, the α-helicoid formation is essential for catecholamine responses but not for (−)-CGP-12177, suggesting that the receptor activated by (−)-CGP12177 binds to G_s, regardless of whether the α-helicoid is intact or not.

The affinity of β*-blockers is conserved across the Arg389Gly polymorphism*

Evidence suggests improvement of heart failure Arg389 patients but hardly of Gly389 patients, during chronic

Table 2 Potencies of (−)-isoprenaline and (−)-CGP12177 and antagonism by (−)-propranolol (200 nM) on recombinant Arg389-β_1-adrenoceptors and Gly389-β_1-adrenoceptors

	Arg389-β-*adrenoceptor*		*Gly389*-β-*adrenoceptor*			
	n		n		%[a]	P
(−)-Isoprenaline						
$-\text{Log}\,EC_{50}M$	13	7.90 ± 0.09	7	9.89 ± 0.13		<0.001
E_{max}[b]		2163 ± 274		60 ± 13	3.0	<0.001
(−)-Propranolol (200 nM)						
$-\text{Log}\,EC_{50}M$	6	5.88 ± 0.07	4	7.65 ± 0.15		<0.001
E_{max}		1995 ± 647		40 ± 14	2.0	<0.001
pK_B		8.72		8.94		
(−)-CGP12177						
$-\text{Log}\,EC_{50}M$	9	7.64 ± 0.12	9	8.18 ± 0.15		<0.001
E_{max}		124 ± 18		67 ± 12	54	<0.02
(−)-Propranolol (200 nM)						
$-\text{Log}\,EC_{50}M$	4	7.45 ± 0.10	5	8.06 ± 0.16		<0.02
E_{max}		109 ± 16		93 ± 24	85	0.62
pK_B		6.44		6.20		

[a](E_{max}Gly389/E_{max}Arg389) × 100.
[b]Maximum agonist effect in pmol mg^{-1} cyclic AMP.
$pK_B = -\log M$(equilibrium dissociation constant) for the (−)-propranolol–receptor complex.

β-adrenoceptor blockade with carvedilol (Perez *et al*., 2003). Furthermore, β_1-adrenoceptor blockade with atenolol (Sofowora *et al*., 2003) and metoprolol (Liu *et al*., 2003) appears to reduce systolic pressure in Arg389 healthy volunteers but not in Gly389 volunteers. These Arg389-specific effects could be due to higher affinity of β-blockers for the Arg389 than Gly389-β_1-adrenoceptors. Our data demonstrating equal affinity of the β-blockers at the two variants are inconsistent with this hypothesis. Instead, cardiac stimulation by endogenous catecholamines may be more pronounced in Arg389 individuals so that the effects of β_1-adrenoceptor blockade are considerably more pronounced than in Gly389 individuals.

The unaltered affinity of (−)-propranolol, estimated from antagonism of the (−)-isoprenaline at the two variants, is consistent with the binding inhibition data.

The Arg389Gly polymorphism reveals marked coupling differences of β_1-adrenoceptors activated by (−)-isoprenaline, but not by (−)-CGP12177

Based on the evidence obtained from Arg389 and Gly389 variants transfected into fibroblasts (Mason *et al*., 1999), it has been proposed that Gly389 disrupts a predicted α-helix within a G_s protein-coupling domain of the β_1-adrenoceptor (Small *et al*., 2003). Mason *et al*. (1999) observed in CHF cells a 66% E_{max} reduction of membrane adenylyl cyclase stimulation by (−)-isoprenaline in Gly389-β_1-adrenoceptors compared to Arg389-β_1-adrenoceptors. In intact CHO cells, we found a 97% E_{max} reduction for (−)-isoprenaline in the Gly389 variant compared to the Arg389 variant, in line with the hypothesis of enhanced G_s coupling of Arg389 β_1-adrenoceptors (Mason *et al*., 1999). Unlike Mason *et al*. (1999), who did not detect differences in the apparent affinity of (−)-isoprenaline between the two variants, we observed an unexpected, nearly 100-fold increase of apparent affinity ($-\log EC_{50}M$) of (−)-isoprenaline at the Gly389 variant compared to the Arg389 variant. The difference of (−)-isoprenaline's apparent affinity between the two laboratories may be related to different host cells expressing the Arg389 and Gly389 variants, as well as to different methods. The nature of the marked increase of (−)-isoprenaline potency at the Gly389 variant, detected in *intact* CHO cells, is not yet understood. Conceivably, still unknown coupling constituents that cause the enhanced (−)-isoprenaline potency at Gly389-β_1-adrenoceptors in *intact* CHO cells are lost in *disrupted* membrane particles of the fibroblasts used by Mason *et al*. (1999). Another difference between our findings and those of Mason *et al*. (1999) is that we did not detect a difference in the basal cyclic AMP levels, while Mason *et al*. (1999) detected a slight increase in basal adenylyl cyclase activity in the Arg389 compared to the Gly389 variant. The discrepancy could be related to the higher density of transfected receptors used by Mason *et al*. (1999) compared to the more physiological density used by us.

In contrast to the profound changes in (−)-isoprenaline responses, the E_{max} of (−)-CGP12177 was only reduced by 46% or by 15% in the presence of (−)-propranolol, and its potency enhanced three-fold at the Gly389 variant compared to the Arg389 variant. From a very weak partial agonist at the Arg389 variant, (−)-CGP12177 became a full agonist with respect to (−)-isoprenaline at the Gly389 variant (Figures 2 and 3). These observations suggest that although the sites activated by each (−)-isoprenaline and (−)-CGP12177 lead to receptor coupling to a G_s/cAMP/PKA-pathway (Kaumann & Lynham, 1997), the coupling mechanism is not identical. While the coupling of Gly389-β_1-adrenoceptors, activated by (−)-isoprenaline, is greatly reduced, compared to coupling of Arg389-β_1-adrenoceptors, coupling of β_1-adrenoceptors, activated by (−)-CGP12177 is only affected to a minor extent by the Arg389Gly polymorphism. The low affinity of (−)-propranolol for the site activated by (−)-CGP12177 appears also unaffected by the Arg389Gly polymorphism. Our results with recombinant β_1-adrenoceptors are in line with the observation in human cardiac myocardium that the effects of (−)-CGP12177 are independent of the Arg389Gly polymorphism (Sarsero *et al*., 2003).

Conclusions

The cAMP signal caused by (−)-isoprenaline, but not by (−)-CGP12177, is markedly reduced at Gly389-β_1-adrenoceptors compared to Arg389-β_1-adrenoceptors. Our results confirm that Arg389 markedly enhances G_s-coupling of β_1-adrenoceptors activated by (−)-isoprenaline, compared to the corresponding activation of Gly389-β_1-adrenoceptors. However, the Arg389Gly polymorphism affects the responses to (−)-CGP12177 considerably less, indicating different modes of G_s-coupling of β_1-adrenoceptors activated by catecholamines and (−)-CGP12177. The affinity of β-blockers and partial agonists is not altered by the Arg389/Gly389 polymorphism.

We are grateful to the British Heart Foundation for support.

References

BAKER, J.G., HALL, I.P. & HILL, S.J. (2003). Agonist actions of β-blockers provide evidence for two agonist-activation sites or conformations of the human β_1-adrenoceptor. *Mol. Pharmacol.,* **63,** 1312–1321.

BUSCHER, R., BELGER, H., EILMES, K.J., TELLKAMP, R., RADKE, J., DHEIN, S., HOYER, P.F., MICHEL, D.C., INSEL, P. & BRODDE, O.E. (2001). *In-vivo* studies do not support a major functional role for the Gly389Arg β_1-adrenoceptor polymorphism in humans. *Pharmacogenetics,* **11,** 199–205.

GOSSEN, M. & BUJARD, H. (1992). Tight control gene expression in mammalian cells by tetracycline responsive promoters. *Proc. Natl. Acad. Sci. U.S.A.,* **89,** 5547–5551.

GRANNEMAN, J.G. (2001). The putative β_4-adrenergic receptor is a novel state of the β_1-adrenergic receptor. *Am. J. Physiol.,* **43,** E199–E202.

JOSEPH, S., LYNHAM, J.A., COLLEDGE, W.E. & KAUMANN, A.J. (2004). Binding of (−)-[^{3}H]-CGP12177 at two sites in recombinant human β_1-adrenoceptors and interaction with β-blockers. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* (in press).

JOSEPH, S., LYNHAM, J.A., MOLENAAR, P., GRACE, A.A., COLLEDGE, W.E. & KAUMANN, A.J. (2003). Intrinsic sympathomimetic activity of (−)-pindolol mediated through a (−)-propranolol-resistant site of the β_1-adrenoceptor in human atrium and recombinant receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **368,** 496–503.

KAUMANN, A.J. (1989). Is there a third heart β-adrenoceptor? *Trends Pharmacol. Sci.,* **10,** 316–320.

KAUMANN, A.J. (1996). CGP12177-induced increase of human atrial contraction through a putative third β-adrenoceptor. *Br. J. Pharmacol.,* **117,** 93–98.

KAUMANN, A.J. (2000). G_s protein-coupled receptors in human heart. In: *The Pharmacology of Functional, Biochemical, and Recombinant Receptor Systems. Handbook of Experimental Pharmacology*. ed. Kenakin, T. & Angus, J.A. Vol. 148, pp. 73–116. Berlin, Heidelberg, New York: Springer.

KAUMANN, A.J. & LYNHAM, J.A. (1997). Stimulation of cyclic AMP-dependent protein kinase in rat atria by (−)-CGP12177 through an atypical β-adrenoceptor. *Br. J. Pharmacol.,* **120,** 1187–1189.

KAUMANN, A.J., LYNHAM, J.A., SARSERO, D. & MOLENAAR, P. (1997). The atypical cardiostimulant β-adrenoceptor is distinct from the β_3-adrenoceptor and is coupled to a cyclic AMP-dependent pathway in rat and human myocardium. *Br. J. Pharmacol.,* **120,** 102.

KONKAR, A.A., ZHAI, Y. & GRANNEMAN, J.G. (2000). β_1-Adrenergic receptors mediate β_3-adrenergic-independent effects of CGP12177 in brown adipose tissue. *Mol. Pharmacol.,* **57,** 252–258.

LEATHERBARROW, R.J. (1992). GraFit version 3.0 Erithacus Software Ltd. (Staines, U.K.).

LEMOINE, H., BILSKI, A. & KAUMANN, A.J. (1989). Xamoterol activates β_1- but not β_2-adrenoceptors in mammalian myocardium: comparison of its affinity for β_1- and β_2-adrenoceptors coupled to the adenylate cyclase in feline and human ventricle and positive inotropic effects. *J. Cardiovasc. Pharmacol.,* **13,** 105–117.

LIU, J., LIU, Z.-Q., TAN, Z.-R., CHEN, X.-P., WANG, L.-S., ZHOU, G. & ZHOU, H.-H. (2003). Gly389Arg polymorphism of β_1-adrenoceptor is associated with the cardiovascular response to metoprolol. *Clin. Pharmacol. Ther.,* **74,** 372–379.

LOWE, M.D., LYNHAM, J.A., GRACE, A.A. & KAUMANN, A.J. (2002). Comparison of the affinity of β-blockers for two states of the β_1-adrenoceptor in ferret ventricular myocardium. *Br. J. Pharmacol.,* **135,** 451–461.

MAQBOOL, A., HALL, A.S., BALL, S.G. & BALMFORTH, A.J. (1999). Common polymorphism of beta1-adrenoceptor: identification and rapid screening assay. *Lancet,* **353,** 897.

MASON, D.A., MOORE, J.D., GREEN, S.A. & LIGGETT, S.B. (1999). A gain-of function polymorphism in a G-protein coupling domain of the human beta1-adrenergic receptor. *J. Biol. Chem.,* **274,** 12670–12674.

MOLENAAR, P., RABNOTT, G., YANG, I., FONG, K.M., SAVARIMUTHU, S.M., LI, L., WEST, M.J. & RUSSELL, F.D. (2002). Conservation of the cardiostimulant effects of (−)-norepinephrine across Ser49Gly and Gly389Arg β_1-adrenergic receptor polymorphisms in human right atrium *in vitro*. *J. Am. Coll. Cardiol.,* **40,** 1275–1282.

MOLENAAR, P., SARSERO, D., ARCH, J.R.S., KELLY, J., HENSON, S.M. & KAUMANN, A.J. (1997). Effects of (−)-RO363 at human atrial β-adrenoceptor subtypes, human cloned β_3-adrenoceptors and rodent intestinal β_3-adrenoceptors. *Br. J. Pharmacol.,* **120,** 165–170.

O'SHAUGHNESSY, K.M., FU, B., DICKERSON, C., THURSTON, D. & BROWN, M.J. (2000). The gain of function variant (R389G) of the β_1-adrenoceptor does not influence blood pressure or response to β-blockade in hypertensive subjects. *Clin. Sci. (Lond.),* **99,** 233–238.

PEREZ, J.M., RATHZ, D.A., PETRASHEVSKAYA, N.N., HAHN, H.S., WAGONER, L.E., SCHWARTZ, A., DORN, G.W. & LIGGETT, S.B. (2003). β_1-Adrenergic receptor polymorphisms confer differential function and predisposition to heart failure. *Nat. Med.,* **9,** 1300–1305.

SANDILANDS, A.J., O'SHAUGHNESSY, K.M. & BROWN, M.J. (2003). Greater inotropic and cyclic AMP responses evoked by noradrenaline through ARG389 β_1-adrenoceptors *versus* Gly389 β_1-adrenoceptors in isolated human atrial myocardium. *Br. J. Pharmacol.,* **138,** 386–392.

SARSERO, D., MOLENAAR, P., KAUMANN, A.J. & FREESTONE, N. (1999). Putative β_4-adrenoceptors in rat ventricle mediate increases in contractile force and cell Ca^{2+}: comparison with atrial receptors and relationship to (−)-[^{3}H]-CGP12177 binding. *Br. J. Pharmacol.,* **128,** 1445–1460.

SARSERO, D., RUSSELL, F.D., LYNHAM, J.A., RABNOTT, G., YANG, I., FONG, K.M., LI, L., KAUMANN, A.J. & MOLENAAR, P. (2003). CGP12177 increases contractile force and hastens relaxation of human myocardial preparations through a propranolol-resistant state of the β_1-adrenoceptor. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **367,** 10–21.

SMALL, K.M., McGRAW, D.W. & LIGGETT, S.B. (2003). Pharmacology and physiology of human adrenergic receptor polymorphisms. *Annu. Rev. Pharmacol. Toxicol.,* **43,** 381–411.

SOFOWORA, G.G., DISHY, V., MUSZKAT, M., XIE, H.G., KIM, R.B., HARRIS, P.A., PRASSAD, H.C., BYRNE, D.W., NAIR, U.B., WOOD, A.J.J. & STEIN, C.M. (2003). A common β_1-adrenergic receptor polymorphism (arg389Gly) affects blood pressure response to β-blockade. *Clin. Pharmacol. Ther.,* **73,** 366–371.

WAGONER, L.E., CRAFT, L.L., ZENGEL, P., McGUIRE, H., RATHZ, D.A., DORN, G.W. & LIGGETT, S.B. (2002). Polymorphism of the β_1-adrenergic receptor predict exercise capacity in heart failure. *Am. Heart J.,* **144,** 840–846.

YABANA, H., WATABANE, H., NARITA, H. & NAGAO, T. (1992). Selective and full beta1-adrenoceptor agonist action of a catechol derivative of denopamine (T-0509) in the guinea-pig cardiac muscle and trachea: comparison with denopamine, xamoterol and isoprenaline. *Br. J. Pharmacol.,* **106,** 335–341.

XIE, H.G., DISKY, V., SOFOWORA, G., KIM, R.B., LANDAU, R., SMILEY, R.M., ZHOU, H.H., WOODS, A.J., HARRIS, P. & STEIN, C.M. (2001). Arg389Gly beta-1 adrenoceptor polymorphism varies in frequency among different ethnic groups but does not alter the response *in vivo*. *Pharmacogenetics,* **11,** 191–197.

(Received February 16, 2004
Accepted February 23, 2004)