Naunyn-Schmiedeberg's Arch Pharmacol (2001) 363 : 87–93
DOI 10.1007/s002100000336

ORIGINAL ARTICLE

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Abolition of (-)-CGP 12177-evoked cardiostimulation in double β_1/β_2-adrenoceptor knockout mice. Obligatory role of β_1-adrenoceptors for putative β_4-adrenoceptor pharmacology

Received: 15 May 2000 / Accepted: 2 August 2000 / Published online: 18 October 2000

Abstract Some β_1- and β_2-adrenoceptor-blocking agents, such as (-)-CGP 12177, cause cardiostimulant effects at concentrations considerably higher than those that antagonise the effects of catecholamines. The cardiostimulant effects of these *non-conventional partial agonists* are relatively resistant to blockade by (-)-propranolol and have been proposed to be mediated through putative β_4-adrenoceptors or through atypical states of either β_1- or β_2-adrenoceptors. We investigated the effects of (-)-CGP 12177 on sinoatrial rate and left atrial contractile force as well as the ventricular binding of (-)-[^{3}H]CGP 12177 in tissues from wild-type, β_2-adrenoceptor knockout and β_1/β_2-adrenoceptor double knockout mice. The cardiostimulant effects of (-)-CGP 12177 were present in wild-type and β_2-adrenoceptor knockout mice but were absent in β_1/β_2-adrenoceptor double knockout mice. Thus, the presence of β_1-adrenoceptors is obligatory for the cardiostimulant effects of (-)-CGP 12177. It appears therefore that an atypical state of the β_1-adrenoceptor contributes to the mediation of the cardiostimulant effects induced by non-conventional partial agonists. Ventricular β_1- and β_2-adrenoceptors, labelled in wild-type with a K_D~0.5 nmol/l (~16 fmol/mg protein), were absent in β_1/β_2-adrenoceptor double knockout mice. However, a high density binding site (~154–391 fmol/mg protein) that did not saturate completely (K_D~80–200 nM) was labelled by (-)-[^{3}H]CGP 12177 in the three groups of mice, being distinct from β_1- and β_2-adrenoceptors, as well as from the site mediating the agonist effects of (-)-CGP 12177.

Key words β_1/β_2-Adrenoceptor double knockout mice · (-)-CGP 12177 · (-)-[^{3}H]CGP 12177 binding site · Cardiostimulation · Obligatory β_1-adrenoceptors · Putative β_4-adrenoceptors

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Introduction

Several blocking agents of β_1- and β_2-adrenoceptors (β_1-AR and β_2-AR) also cause cardiostimulant effects at concentrations that greatly exceed their affinity for these receptors. The agonist effects of these agents are usually smaller than those of catecholamines and it has been suggested that these *non-conventional partial agonists* mediate their effects through a putative β_4-adrenoceptor (β_4-AR; Kaumann 1997). Although its pharmacology resembles that of β_3-adrenoceptor (β_3-AR; Kaumann 1989), cardiostimulation through the putative β_4-AR is preserved in mice with disrupted β_3-AR gene (β_3-AR knockout), indicating involvement of a distinct receptor (Kaumann et al. 1998). It is possible, however, that either β_1-AR or β_2-AR or both are involved in the cardiostimulant effects of non-conventional partial agonists, although this would be inconsistent with classical pharmacological principles (Lowe et al. 1999) because agonist potency is considerably lower than receptor affinity and because the effects are relatively resistant to blockade by the β_1/β_2-AR antagonist propranolol. Interestingly, in host cells expressing high densities of recombinant receptors, (±)-CGP 12177 can enhance cellular cyclic AMP through both β_1-AR and β_2-AR at concentrations approximately 100-fold greater than those that half-saturate (Pak and Fishman 1996) and block these receptors. An equivalent dissociation between high-affinity blockade and lower-potency agonist effects had been observed previously in three different feline car-

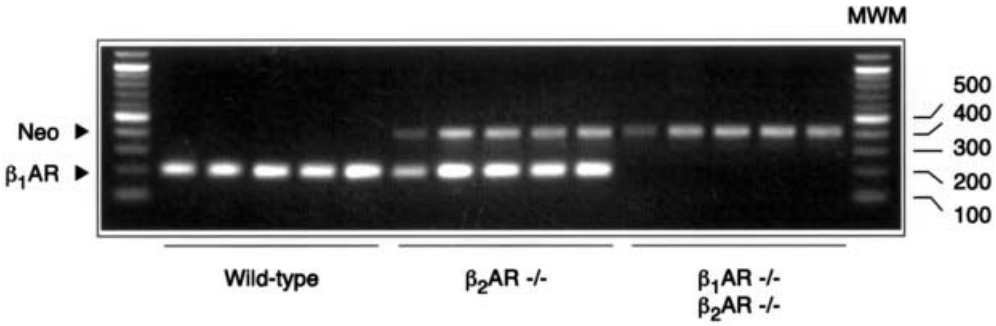

Fig. 1 Genotyping of β_2-adrenoceptor knockout (β_2-AR-/-), β_1/β_2-adrenoceptor double knockout (β_1AR-/- β_2AR-/-) and wild-type mice. Genomic DNA of either genotype was amplified using two sets of primers specific for the neomycin resistance gene (*Neo*) and the endogenous β_1-adrenoceptor gene (β_1-AR). The expected length of the PCR products is 200 bp for the β_1-adrenoceptor gene and 404 bp for the neomycin resistance gene

diac regions with (±)-CGP 12177 (Kaumann 1983), and this ligand has been used increasingly as a prototype non-conventional partial agonist (Kaumann and Molenaar 1996; Malinowska and Schlicker 1996; Preitner et al. 1998; Sarsero et al. 1999; Oostendorp et al. 2000). (-)-[^{3}H]CGP 12177 labels a cardiac site, presumably the putative β_4-AR, for which (-)-CGP 12177 and other non-conventional partial agonists compete with affinities similar to their corresponding cardiostimulant potencies but for which (-)-propranolol has low affinity (Kaumann et al. 1998; Sarsero et al. 1998a, 1999).

To assess whether β_1-AR or β_2-AR are involved, we have studied the cardiostimulant effects of (-)-CGP 12177 and cardiac binding of (-)-[^{3}H]CGP 12177 in mice that do not express β_2-AR (β_2-AR knockout; Chruscinski et al. 1999) or lack both β_1-AR and β_2-AR (β_1/β_2-AR double knockout; Rohrer et al. 1999). Cardiostimulant effects of (-)-CGP 12177 are present in β_2-AR knockout but are abolished in the β_1/β_2-AR double knockout. Surprisingly, the density and affinity of binding sites for (-)-[^{3}H]CGP 12177, formerly attributed to putative β_4-adrenoceptors (Sarsero et al. 1999) and found in the ventricles of wild-type mice, remained in the ventricles from β_1/β_2-AR double knockout and β_2-AR knockout mice.

A preliminary account of this work was presented at the joint meeting of the Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists and the British Pharmacological Society, Melbourne, Australia (Kaumann et al. 2000).

Materials and methods

Mice. β_2-AR knockout and β_1/β_2-AR double knockout mice of either sex, aged 20±1 months, were generated in Dr. Brian Kobilka's laboratory as described (Chruscinski et al. 1999; Rohrer et al. 1999) and propagated in our laboratory. Age-matched NMRI mice of either sex were used as wild-type mice.

Genotyping. The identification of knockout and wild-type mice (Fig. 1) was carried out by amplification of transgene-specific sequences from genomic DNA by PCR. Genomic DNA was isolated from mouse tail biopsies as described previously (Gordon 1993). One-hundred nanograms of DNA was used with two sets of oligonucleotide primers to amplify both the neomycin resistance gene and the endogenous β-adrenoceptor gene (Neo forward 3'TATCAGGACATAGCGTTGG5', Neo reverse 3'ATAGAGCCCACCGCATCC5', β_1-AR forward 3'ATGGCCTTCGTGTACCTGC5', β_1-AR reverse 3'AGAGCCACGAGGCGCGAC5'). Ten microliters of the PCR reaction was then separated on a 1.5% agarose gel and stained with ethidium bromide.

Isolated atria. Mice were killed by neck dislocation and the hearts immediately taken out and dissected in oxygenated solution at room temperature containing (mmol/l): NaCl 119, $NaHCO_3$ 25, KCl 4, KH_2PO_4 1.2, $MgCl_2$ 1, $CaCl_2$ 1.8, glucose 10 and Na-pyruvate 2. Right atria and left atria were carefully dissected. Spontaneously beating right atria or left atria, paced at 2 Hz, were set up in pairs at 37°C in a 50-ml organ bath (Blinks 1965) containing above solution with one tissue from a β_2-AR knockout and the other from a β_1/β_2-AR double knockout mouse. Right atrial beating rate and left atrial force were recorded as described (Kaumann et al. 1998). Left atria were exposed successfully without washout to 3-isobutyl-1-methylxanthine (IBMX; 3 μmol/l; Kaumann and Lynham 1997; Kaumann et al. 1998), (-)-CGP 12177 (1 μmol/l), prazosin (1 μmol/l), (-)-isoprenaline (400 μmol/l) and dibutyryl cyclic AMP (1 mmol/l) as shown in the representative experiment of Fig. 2. Right atria were exposed successfully to (-)-CGP 12177 (1 μmol/l), prazosin (1 μmol/l), (-)-isoprenaline (400 μmol/l) and IBMX (20 μmol/l), allowing for steady-state responses after each drug before administering the next drug.

Binding assay. During dissection of the hearts and after cutting away valves and great vessels, the ventricles were freeze-clamped in liquid nitrogen. The ventricles were homogenised in ice-cold assay buffer containing 50 mmol/l Tris HCl, 5 mmol/l EGTA, 1 mmol/l EDTA, 4 mmol/l $MgCl_2$, 1 mmol/l ascorbic acid and 0.5 mmol/l phenylmethylsulfonyl fluoride, pH 7.4, centrifuged for 15 min at 50,000 *g* at 4°C and the pellet resuspended in 15 volumes of ice-cold assay buffer. For saturation binding to β_1-AR, β_2-AR and putative β_4-AR of ventricular membranes 0.01~200 nmol/l (-)-[^{3}H]CGP 12177 (specific activity 44.5 Ci/mmole) was used in the presence and absence of 20 μmol/l (-)-CGP 12177 to define non-specific binding. All assays were in the presence of 100 μmol/l GTP and at 37°C. The experiments were analysed for one or two binding sites, one with high (H) affinity (i.e. β_1-AR + β_2-AR because β_1-AR and β_2-AR have similar affinity for (-)-[^{3}H]CGP 12177; Nanoff et al. 1987) and another with low (L) affinity (i.e. putative β_4-AR; Kaumann et al. 1998; Sarsero et al. 1998a, 1999) with the following equation:

$$Beq = \sum_i f_i \; B_{max} . \; \log C / (\log K + \log C) \qquad (1)$$

where f_i, i=1 or 2 for one or two site fits, B_{max} is the density of receptors, K is the equilibrium dissociation constant of radioligand and C is the concentration of (-)-[^{3}H]CGP 12177.

Inhibition of binding of (-)-[^{3}H]CGP 12177 (68–71 nmol/l) was assayed by incubating competing ligands for 120 min. Protein was determined using bovine serum albumin as standard (Lowry et al. 1951).

Statistics. All data are expressed as means ± SEM. Significance between differences was assessed with Student's *t*-test or one-way ANOVA followed by the Bonferroni method for multiple comparisons using InStat (GraphPad Software, San Diego, Calif., USA). Radioligand binding data were analysed by non-linear regression for one or two sites and the fits statistically compared by calculating the *F*-ratio using PRISM. All statistical programs were from GraphPad Software.

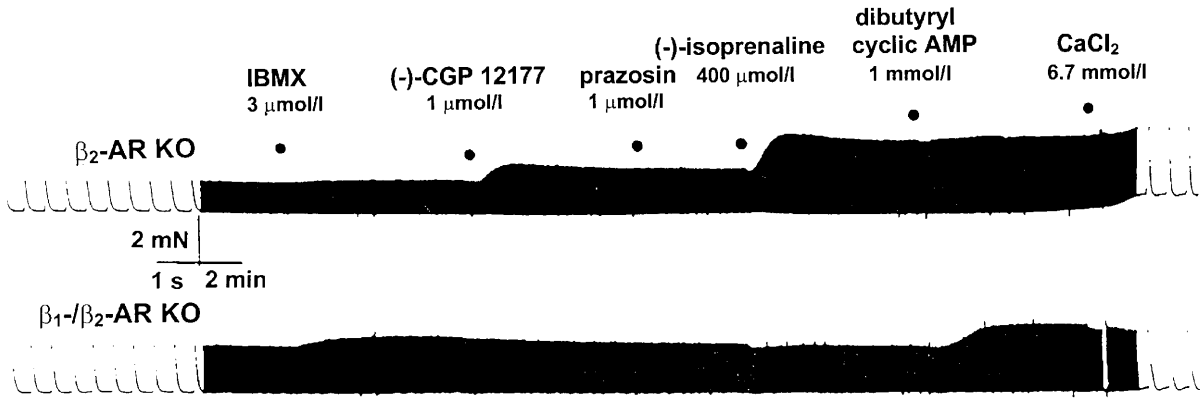

Fig. 2 Comparison of the effects of IBMX (3 μmol/l), (-)-CGP 12177 (1 μmol/l), (-)-isoprenaline (400 μmol/l) and dibutyryl cyclic AMP (1 mmol/l) on a left atrium from a β_2-AR knockout mouse (β_2-AR KO) and a β_1/β_2-AR double knockout (β_1/β_2-AR KO) mouse set up in the same organ bath. Fast and slow speed *tracings* are shown. Notice the positive inotropic effects of (-)-CGP 12177 and (-)-isoprenaline in the β_2-AR knockout atrium but their complete absence in the β_1/β_2-AR double knockout atrium. Both atria responded to IBMX and the β_1/β_2-AR double knockout atrium also responded to dibutyryl cyclic AMP. The experiment was terminated by raising the $CaCl_2$ concentration to 6.7 mmol/l

Drugs. (-)-CGP 12177 ((-)-4-3(tertiarybutylamino-2-hydroxy-propoxy)benzimidazol-2-one hydrochloride; a gift of Dr. Jonathan Arch) and (±)-carvedilol hydrochloride were from SmithKline Beecham (Harlow, UK); CGP 20712A, (2-hydroxy-5(2-((2-hydroxy-3-(4-((1-methyl-4-trifluoromethyl) 1*H*-imidazole-2-yl)-phenoxy) propyl) amino) ethoxy)-benzamide monomethane sulphonate; a gift of Alexandra Sedlacek), (±)-cyanopindolol hydrochloride and (-)-pindolol tartrate were from Novartis (Basel, Switzerland); IBMX (3-isobutyl-1-methylxanthine), dibutyryl cyclic AMP, (-)-propranolol hydrochloride and (-)-isoprenaline hydrochloride were from Sigma; prazosin was from Pfizer (Sandwich, Kent, UK); (-)-bupranolol was a gift of Dr. Klaus Sandrock (Sanol-Schwarz, Monheim, Germany); (+)-isoprenaline (+)-bitartrate was from Sterling-Winthrop Research Institute (Rensselaer, N.Y., USA); (±)-carazolol was from Boehringer Mannheim (Germany).

Results

Cardiostimulant effects of (-)-CGP 12177 are absent in atria from β_1/β_2-AR double knockout but present in atria from β_2-AR knockout mice

Increases in contractile force evoked by (-)-CGP 12177 were observed in left atria from both wild-type and β_2-AR knockout but were absent in atria from β_1/β_2-AR double knockout mice (Figs. 2, 3). As observed previously in wild-type and β_3-AR knockout mice (Kaumann et al. 1998), the response to (-)-CGP 12177 was a fraction of the response to (-)-isoprenaline in left atria from wild-type and β_2-AR knockout mice (Figs. 2, 3). Similarly to previous work (Rohrer et al. 1999), (-)-isoprenaline failed to enhance contractions of left atria from β_1/β_2-AR double knockout mice (Figs. 2, 3). Prazosin did not affect the responses to (-)-CGP 12177 in both wild-type and β_2-AR knockout mice, excluding an involvement of α_1-adrenoceptors. Left atria from β_1/β_2-AR knockout mice responded with enhanced contractile force to dibutyryl cyclic AMP (Figs. 2, 3), consistent with an intact functional pathway downstream from adenylyl cyclase. Because this pathway was fully activated by (-)-isoprenaline in left atria from both wild-type and β_2-AR knockout mice, dibutyryl cyclic AMP did not cause additional effects in these tissues (Figs. 2, 3).

Increases in sinoatrial rate by both (-)-CGP 12177 and (-)-isoprenaline were absent in right atria from β_1/β_2-AR double knockout mice but present in atria from β_2-AR knockout mice (Fig. 4). As seen previously in right atria from wild-type mice (Kaumann et al. 1998), (-)-CGP 12177 caused significant increases in sinoatrial beating rate in right atria from β_2-AR knockout mice. The (-)-CGP 12177-evoked tachycardia was resistant to blockade by prazosin, excluding involvement of α_1-adrenoceptors. Similarly to previous work in β_1-AR knockout (Rohrer et al. 1996) and β_1/β_2-AR double knockout (Rohrer et al. 1999) mice,

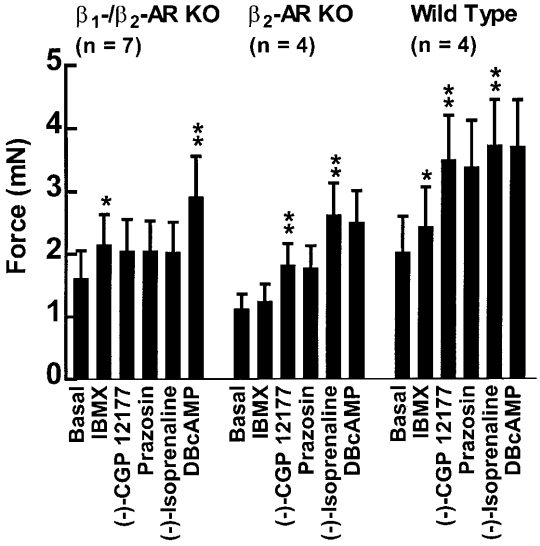

Fig. 3 Comparison of the positive inotropic effects of IBMX (3 μmol/l), (-)-CGP 12177 (1 μmol/l), (-)-isoprenaline (400 μmol/l) and dibutyryl cyclic AMP (1 mmol/l) in left atria from wild-type, β_2-AR knockout (β_2-AR KO) and β_1/β_2-AR double knockout (β_1/β_2-AR KO) mice. **P*<0.05 with respect to basal, ***P*<0.05 with respect to IMBX

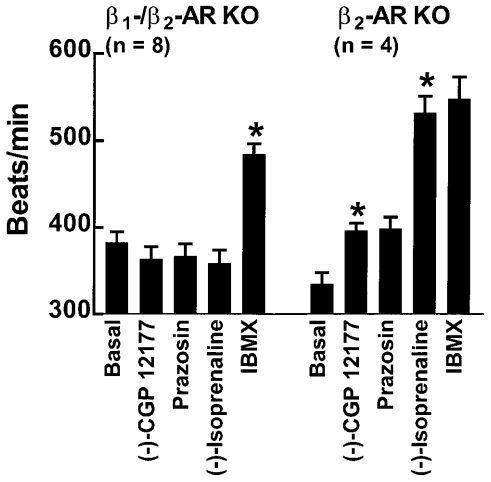

Fig. 4 Comparison of the positive chronotropic effects of (-)-CGP 12177 (1 μmol/l), (-)-isoprenaline (400 μmol/l) and IBMX (20 μmol/l) on spontaneously beating right atria from β_1/β_2-AR double knockout (β_1/β_2-AR KO) mice and β_2-AR knockout (β_2-AR KO) mice. *P<0.05 with respect to basal

(-)-isoprenaline-evoked tachycardia was abolished in β_1/β_2-AR knockout (Fig. 4). IBMX enhanced sinoatrial beating rate in right atria from β_1/β_2-AR double knockout mice, presumably through accumulation of cyclic AMP, indicating that this pathway is intact. Through activation of β_1-AR (-)-isoprenaline increased maximally sinoatrial rate in atria from β_2-AR knockout that precluded further tachycardia by IBMX (Fig. 4).

Binding of (-)-[^{3}H]CGP 12177 to a high capacity site in ventricular membranes from wild-type, β_2-AR knockout and β_1/β_2-AR double knockout mice

(-)-[^{3}H]CGP 12177 bound to two populations of sites in membranes from wild-type and β_2-AR knockout mice but only to one population in membranes from β_1/β_2-AR double knockout mice (Fig. 5; Table 1). In membranes from wild-type and β_2-AR knockout mice we found a high-affinity (K_D~0.5 nM) saturable population of low density (16 fmol/mg protein and 12 fmol/mg protein) corresponding to β_1-AR plus β_2-AR and β_1-AR, respectively. In all three groups of mice we identified a high-density population of ventricular sites (154–391 fmol/mg protein) that did not saturate completely due to constraints in the use of high (-)-[^{3}H]CGP 12177 concentrations (see extrapolated sigmoid binding curves of Fig. 5), precluding precise estimates of K_D and B_{max}. However, the data fitted reasonably well to one population of sites, so that approximate K_i-values between 80–200 nM could be estimated. For unknown reasons the density of these ventricular sites appeared to be larger in β_2-AR knockout than in the wild-type and β_1/β_2-AR double knockout mice (Fig. 5; Table 1).

(±)-Cyanopindolol (pK_i 5.68±0.13), (-)-isoprenaline (pK_i 3.74±0.18) and (+)-isoprenaline (pK_i 3.94±0.18) competed with (-)-[^{3}H]CGP 12177 for binding in ventricular membranes prepared from β_1/β_2-AR double knockout mice (pK_i-values determined from three independent assays carried out in triplicate). (-)-[^{3}H]CGP 12177 binding to these membranes was also inhibited (% inhibition be-

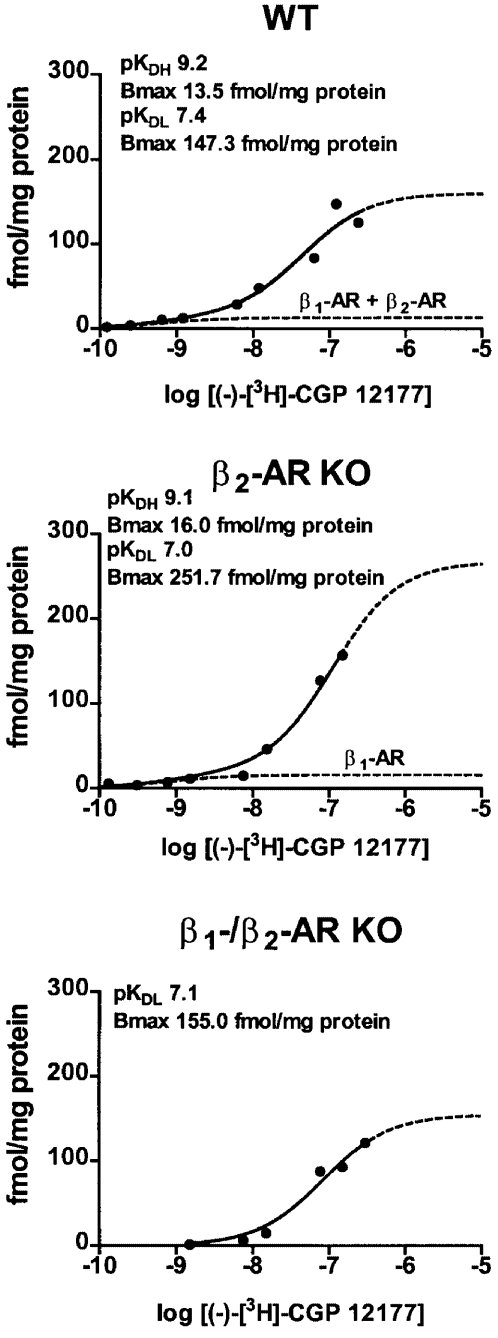

Fig. 5 Saturation binding experiments showing specific binding of (-)-[^{3}H]CGP 12177 (0.1–240 nmol/l) to ventricular membranes prepared from a wild-type (*WT*), a β_2-AR knockout (β_2-AR KO) and a β_1/β_2-AR double knockout (β_1/β_2-AR KO) mouse. *Solid lines* show non-linear fits of data, with extrapolations shown by *broken lines* using estimates of K_D and B_{max} determined from Eq. 1. *Broken lines* in WT and β_2-AR knockout also show theoretical fits for β_1-AR + β_2-AR and β_1-AR, respectively, from Eq. 1

Table 1 Comparison of saturation binding of (-)-[^{3}H]CGP 12177 to ventricular membranes from wild-type (*WT*), β_2-AR knockout (β_2-AR KO) and β_1/β_2-AR double knockout (β_1/β_2-AR KO) mice

	WT	β_2-AR KO	β_1/β_2-AR KO
n	3	4	4
n_H	0.73±0.07	0.76±0.02	0.94±0.06
$-\log K_{DH}$	9.20±0.06	9.34±0.24	–
$-\log K_{DL}$	6.98±0.20	6.67±0.11*	7.11±0.13
B_{maxH} (fmol/mg protein)	15.6 ±2.4	12.1 ±2.3	–
B_{maxL} (fmol/mg protein)	212.4±19.8	390.9±86.2*	154.2 ±14.5

*P<0.04 with respect to β_1/β_2-AR KO

tween parentheses) by 10 μmol/l of (-)-pindolol (48.8± 12.4), (±)-carazolol (37.5±6.6), (±)-carvedilol (37.1±6.7), (-)-bupranolol (24.7±5.2), (-)-propranolol (31.7±8.6) and CGP 20712A (–7.3±14.0).

Discussion

When both β_1-AR and β_2-AR are absent, (-)-CGP 12177 fails to produce atrial cardiostimulant effects which were observed in atria from β_2-AR knockout and wild-type mice. Consequently, β_1-ARs have an obligatory role in the mediation of (-)-CGP 12177-evoked cardiostimulation. The simplest explanation of these findings is that the cardiostimulant effects of (-)-CGP 12177 are mediated through an atypical state of the β_1-AR. Previously the effects of CGP 12177 and other non-conventional partial agonists had been suggested to be mediated through putative β_4-AR (Kaumann 1997; Kaumann and Molenaar 1997; Molenaar et al. 1997). Alternatively, in light of the results with recombinant β_1-AR of Pak and Fishman (1996), the cardiostimulant effects of CGP 12177 have been interpreted to be due to an atypical state of the β_1-AR (Freestone et al. 1999; Kompa and Summers 1999; Lowe et al. 1999; Sarsero et al. 1999; Konkar et al. 2000; Oostendorp et al. 2000). Our data from β_1/β_2-AR double knockout mice prove the latter, indicating that the pharmacology of putative β_4-ARs is not due to a novel gene, as envisioned previously (Kaumann et al. 1998), but to an atypical state of the β_1-AR. We have previously argued that this state has lower affinity for (-)-CGP 12177 than the high-affinity β_1-AR state through which (-)-CGP 12177 blocks the cardiostimulant effects of catecholamines (Lowe et al. 1999). The argument is supported by the observation in several regions of feline heart that the cardiostimulant potency of CGP 12177 is at least 100 times lower than its blocking potency against the effects of (-)-isoprenaline (Kaumann 1983).

CGP 12177 also has agonist activity in mouse brown fat, mediated through both β_3-AR and an atypical β-adrenoceptor, suggested to be a putative β_4-AR (Galitzky et al. 1997; Preitner et al. 1998). CGP 12177 has been shown to stimulate adenylyl cyclase in mouse brown fat with high potency through atypical β-adrenoceptors but with low potency through β_3-AR. Konkar et al. (2000) have recently demonstrated that the high- and low-potency components of the agonist effects of CGP 12177 in brown fat are absent in β_1-AR knockout and in β_3-AR knockout, respectively. Thus, as in heart, the atypical β-adrenoceptor in brown fat, previously suggested to be a putative β_4-AR (Galitzky et al. 1997; Preitner et al. 1998), is actually an atypical state of the β_1-AR (Konkar et al. 2000).

In contrast to its relatively low potency as a positive chronotropic and inotropic agent, (-)-CGP 12177 also elicits arrhythmias with high potency. (-)-CGP 12177 can produce ventricular arrhythmias and abbreviation of action potentials in perfused ferret hearts; (-)-noradrenaline produces similar but less potent effects mediated through β_1-AR (Lowe et al. 1998). Moreover, in murine ventricular myocytes (-)-CGP 12177 causes arrhythmic Ca^{2+} transients and is approximately 40 times more potent than (-)-isoprenaline for this effect (Freestone et al. 1999). These arrhythmogenic effects of (-)-CGP 12177 are resistant to blockade by (-)-propranolol (Lowe et al. 1998; Freestone et al. 1999; Sarsero et al. 1999) but blocked by (-)-bupranolol (Freestone et al. 1999), which are characteristics of putative β_4-AR (Kaumann 1989, 1997). The arrhythmic potency of (-)-CGP 12177 is, however, considerably higher than its cardiostimulant potency and matches its affinity for β_1-AR, suggesting mediation through a high-affinity β_1-AR state (Freestone et al. 1999) that is clearly different from a low-affinity state that mediates positive inotropic and chronotropic effects of (-)-CGP 12177 in a variety of species from mouse (Kaumann et al. 1998) to man (Kaumann 1996; Kaumann and Molenaar 1997). It is therefore plausible that different states or conformations of the β_1-AR can mediate (1) cardiostimulant effects of catecholamines, (2) positive inotropic and chronotropic effects of (-)-CGP 12177 and (3) arrhythmogenic effects of (-)-CGP 12177 and (4) blockade by (-)-CGP 12177 of the cardiostimulant effects of catecholamines.

Both positive inotropic and chronotropic effects of (-)-CGP 12177, mediated through β_1-AR, were observed in atria from β_2-AR knockout mice, but appeared slightly smaller than in atria from wild-type mice (Kaumann et al. 1998). This would suggest that β_2-AR could contribute to a small extent to the positive inotropic effects of (-)-CGP 12177, which would be in line with the relatively small increases in cellular cyclic AMP, compared to those mediated through recombinant β_1-AR, elicited by (±)-CGP 12177 through transfected recombinant β_2-AR (Pak and Fishman 1996). If residual cardiostimulant effects of (-)-CGP 12177 are mediated through a (-)-propranolol-resistant state of the β_2-AR, these effects would be expected to be observed in β_1-AR knockout mice, which remains to be investigated. The contribution of murine β_2-AR to the cardiostimulant effects of (-)-CGP 12177 is, however, unlikely because their coupling to G_i protein prevents mediation of cardiostimulant effects (Xiao et al. 1999). Furthermore, (-)-adrenaline does not elicit increases in atrial contractility through β_2-AR, not even after inactivation of G_i protein with pertussis toxin (Oostendorp and Kaumann

2000), further casting doubt on the functional role of murine atrial β_2-ARs.

In wild-type mice (-)-[^{3}H]CGP 12177 labelled a high-affinity (K_D~0.5 nM), relatively low-density binding population (16 fmol/mg protein), consistent with β_1-AR plus β_2-AR, similar to that recently observed with (-)-[^{125}I]iodocyanopindolol (11 fmol/mg protein; Heubach et al. 1999). As expected, this binding population was absent in the ventricle of β_1/β_2-AR double knockout mice. On the other hand, binding sites for (-)-[^{3}H]CGP 12177 with a K_D~100 nM, which were non-stereoselective and had low affinity for non-conventional partial agonists, were present in wild-type, β_2-AR knockout and β_1/β_2-AR double knockout mice. Because (-)-CGP 12177-evoked cardiostimulation is abolished in atria from β_1/β_2-AR double knockout mice, it appears that these binding sites are not involved in the cardiostimulant effects of (-)-CGP 12177. Similar binding sites with a K_D~100 nM for (-)-[^{3}H]CGP 12177 and low affinity for non-conventional partial agonists have been reported previously in atrium from rat and man (Sarsero et al. 1998a, 1998b), and in ventricle from man (Sarsero et al. 1998b), rat (Sarsero et al. 1999), pig and ferret (Kaumann and Lynham, unpublished observations). These binding sites were also considered not to mediate the cardiostimulant effects of non-conventional partial agonists because their affinities were lower (K_i> 10 μmol/l) than their corresponding cardiostimulant potencies (EC_{50}<1 μmol/l; Sarsero et al. 1999). In rat atrium and ventricle, the non-conventional partial agonists, (±)-cyanopindolol, (-)-pindolol and (±)-carazolol, but not (-)-[^{3}H]CGP 12177 could distinguish between binding sites corresponding to functional (receptors) and non-functional sites (Sarsero et al. 1999).

We conclude that β_1-AR are obligatory for the mediation of cardiostimulant effects of the non-conventional partial agonist (-)-CGP 12177. It appears likely that qualitatively different effects mediated through β_1-AR are due to different conformations of this receptor. We suggest that the agonist effects of (-)-CGP 12177 might be produced through interaction with an allosteric site at the β_1-adrenoceptor.

Acknowledgements We thank Dr. Brian Kobilka (Stanford University) for kindly donating knockout mice. This work was supported by the British Heart Foundation (A.J.K.) and the NHMRC Australia (P.M).

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