British Journal of Pharmacology (1999) 128, 1399–1406
http://www.stockton-press.co.uk/bjp

Desensitization and resensitization of β_1- and putative β_4-adrenoceptor mediated responses occur in parallel in a rat model of cardiac failure

[1]Andrew R. Kompa & *,[1]Roger J. Summers

[1]Department of Pharmacology, Monash University, Wellington Road, Clayton, Victoria, 3168, Australia

1 Cardiostimulant effects of the non-conventional partial agonist, CGP 12177A, are mediated by a receptor distinct from the β_3-adrenoceptor and termed the putative β_4-adrenoceptor. Using a rat model of cardiac failure, induced by myocardial infarction (MI), we compared the desensitization and resensitization of responses to CGP 12177A with those to isoprenaline and RO 363 in left (LA) and right atria (RA). We also examined the ability of β-adrenoceptor antagonists to block responses to CGP 12177A.

2 MI reduced the maximum inotropic response to isoprenaline by 48% (sham 4.1 ± 0.6 mN, $n=10$; MI 2.1 ± 0.4 mN, $n=8$, $P<0.02$), RO 363 by 61% (sham 4.2 ± 0.5 mN, $n=10$; MI 1.8 ± 0.3 mN, $n=8$, $P<0.005$) and CGP 12177A by 49% (sham 1.4 ± 0.1 mN, $n=5$; MI 0.7 ± 0.2 mN, $n=7$, $P<0.05$) in electrically stimulated LA. MI also reduced the sensitivity to isoprenaline (pEC_{50}: sham 8.79 ± 0.08, $n=10$; MI 8.30 ± 0.10, $n=8$; $P=0.001$) and RO 363 (pEC_{50}: sham 8.69 ± 0.07, $n=10$; MI 8.33 ± 0.10, $n=8$; $P<0.01$). The maximum chronotropic responses to isoprenaline, RO 363 and CGP 12177A in RA were unaffected.

3 Pertussis toxin treatment ($10\ \mu g\ kg^{-1}$, i.p.) restored the maximum inotropic response and sensitivity to isoprenaline (sham 3.5 ± 0.5 mN, $n=9$; MI 3.2 ± 0.6 mN, $n=11$, $P=0.702$) and CGP 12177A (sham 1.6 ± 0.3 mN, $n=6$; MI 1.9 ± 0.4 mN, $n=7$, $P=0.537$) in MI animals to levels similar to those in the sham group.

4 CGP 20712A (pK_B: LA 6.7 ± 0.2, $n=6$; RA 7.1 ± 0.1, $n=4$), ICI 118,551 (pK_B: LA 6.4 ± 0.1, $n=5$; RA 6.3 ± 0.1, $n=6$), propranolol (pK_B: LA 6.6 ± 0.1, $n=5$; RA 6.8 ± 0.1, $n=6$) and bupranolol (pK_B: LA 7.2 ± 0.1, $n=6$; RA 7.7 ± 0.1, $n=8$), showed moderate affinity for the putative β_4-adrenoceptor.

5 Desensitization after MI and resensitization (after pertussis toxin treatment) to isoprenaline and CGP 12177A therefore occur in parallel, suggesting that the β_1- and putative β_4-adrenoceptor use the same signalling pathway. Antagonist affinity studies confirmed that drugs acting at β_1-adrenoceptors also interact with putative β_4-adrenoceptors with approximately 100 times lower affinity. We suggest that CGP 12177A produces its cardiac effects by interacting with a low affinity state of the β_1-adrenoceptor.

Keywords: β_1-adrenoceptor; putative β_4-adrenoceptor; CGP12177A; isoprenaline; RO 363; cardiac failure; rat atrium; pertussis toxin

Abbreviations: DR, dose ratio; MI, myocardial infarction; ANOVA (RM ANOVA), repeated measures

Introduction

There is abundant evidence for the existence of β_1- and β_2-adrenoceptors from functional (Carlsson *et al*., 1972), binding (Minneman *et al*., 1979) and autoradiographic studies (Summers *et al*., 1985), and by gene (Frielle *et al*.,1987; Caron *et al*., 1988) and protein (Benovic *et al*., 1984; Cubero & Malbon, 1984) expression. There is also evidence for a third functional β-adrenoceptor in heart, distinct from the β_3-adrenoceptor found in gut, white and brown adipose tissue, which has been termed the 'putative β_4-adrenoceptor' (Kaumann & Molenaar, 1997). This receptor is stimulated by non-conventional partial agonists, such as CGP 12177A. At low concentrations these compounds are known to act as antagonists at β_1- and β_2-adrenoceptors, but may at higher concentrations act as agonists activating the putative β_4-adrenoceptor (Kaumann, 1989). The cardiostimulant effects of CGP 12177A are unaffected by propranolol (200 nM) and are blocked by bupranolol (pK_B 6.4–6.8) and CGP 20712A (pK_B 6.3–6.4) (Kaumann & Molenaar, 1996). Furthermore, CGP 12177A increases cyclic AMP levels (Kaumann *et al*., 1997) and causes increased activity of cyclic AMP-dependent protein kinase, and its effects are potentiated by the phosphodiesterase inhibitor IBMX (Kaumann & Lynham, 1997). This suggests Gs-protein coupling of the putative β_4-adrenoceptor to adenylate cyclase.

[^{3}H]CGP 12177A has also been used as a radioligand for the putative β_4-adrenoceptor in rat atrium (Sarsero *et al*., 1998). However in spite of a wealth of evidence from functional and, more recently, binding studies the putative β_4-adrenoceptor remains to be cloned. One possible interpretation of this is that the site under study is an alternative affinity state of a known receptor. For example, in transfected CHW-cells expressing human β_1-adrenoceptors, CGP 12177A behaves as an agonist with the same potency seen in heart at putative β_4-adrenoceptors (Kaumann & Molenaar, 1996), and is able to stimulate adenylate cyclase suggesting that this pathway also couples to Gs (Pak & Fishman, 1996). The aim of this study was to examine the desensitization of adrenergic responses in the rat model of cardiac failure to stimulation of the β_1- or putative β_4-adrenoceptor to determine whether changes in sensitivity could occur independently for each receptor subtype. The results show that desensitization of both β_1- and

*Author for correspondence;
E-mail: Roger.Summers@med.monash.edu.au

putative β_4-adrenoceptor mediated responses occur exactly in parallel, as does the resensitization induced by prior administration of pertussis toxin. The results suggest that either both 'receptors' utilize identical signalling pathways or that the 'putative β_4-adrenoceptor' represents a low affinity binding site of the β_1-adrenoceptor.

Methods

Rat model of cardiac failure

Sprague-Dawley rats were anaesthetized with Alfaxan (1.5 ml kg^{-1}, i.v.), intubated and placed on a respirator. A left thoractomy was performed, the heart exteriorized and the left coronary artery ligated (MI) with a nylon suture, the chest was closed and animals allowed to recover. Sham animals were treated the same except the suture was not tied. Four weeks after surgery, animals were anaesthetized with 20% O_2 in CO_2, decapitated, and tissue weights and infarct size determined. The whole left ventricle (LV) was dissected and the infarct size determined by tracing the whole LV and infarcted area under a transparent plate for both the exterior (epicardial) and interior (endocardial) surfaces. Surface areas were calculated using an image-analysis system (MCID; Imaging Research, St Catharines, Ontario, Canada) and the infarct size for the epicardial and endocardial scar area was expressed as a percentage of the LV (Gu *et al*., 1998; Kompa *et al*., 1999). Tissues from animals with an infarct size >45% of the LV were used in this study, animals with smaller infarcts were excluded. This level of ischaemic damage produces right ventricular hypertrophy and increased lung weight, as well as haemodynamic changes resulting in reduced and sustained systolic blood pressure (Kompa *et al*., 1999). Lung and heart tissues from animals used in the functional studies were weighed and expressed as a ratio of body weight (Table 1).

Functional studies

Four weeks after surgery sham and MI animals were reserpinized (1 mg kg^{-1}, i.p.) 18 h before functional studies were performed. Hearts were removed, placed in warm Krebs bicarbonate buffer (composition mM): NaCl 118.4; KCl 4.7; $NaHCO_3$ 25; $MgSO_4$ 1.2; Na_2HPO_4 1.2; $CaCl_2$ 1.9; Glucose 11.7) and dissected to obtain left and right atria. These were mounted in organ baths under $0.5 \times g$ tension, at 37°C and aerated with carbogen. Left atria were paced at 2 Hz with square-wave pulses of 5 msec duration at 1.5 times threshold voltage using a Grass SD9 stimulator. Right atria were allowed to beat spontaneously. Tissues were incubated with 50 μM phenoxybenzamine for 30 min, to block actions at other receptors, this was followed by eight washes over 30 min and a further period of equilibration. Inotropic and chronotropic responses to the non-selective β-adrenoceptor agonist, isoprenaline, the selective β_1-adrenoceptor agonist, RO 363, and the putative β_4-adrenoceptor agonist, CGP 12177A, were measured isometrically using a Grass force-displacement transducer (FTO3C) and a MacLab system. The effects of isoprenaline and RO 363 were examined in one group of sham ($n=10$) and MI ($n=8$) animals, and the effects of CGP 12177A examined in another group (sham $n=5$; MI $n=7$).

In separate experiments sham and MI animals were treated with pertussis toxin (10 μg kg^{-1} i.p.) 3 days prior to experimentation and cumulative concentration-response curves to isoprenaline and CGP 12177A were constructed in left and right atria as described above. The effects of isoprenaline and RO 363 were examined in one group of sham ($n=10$) and MI ($n=11$) animals, and the effects of CGP 12177A examined in another group (sham $n=6$; MI $n=7$).

Further comparisons between β_1- and β_4-adrenoceptors were made in left and right atria of unoperated rats. Tissues were mounted in the organ bath as previously described and cumulative concentration-response curves to CGP 12177A were constructed in the absence and presence of the following β-adrenoceptor antagonists: CGP 20712A (β_1); ICI 118,551 (β_2); propranolol (β_1 and β_2); bupranolol (β_1, β_2 and β_4). pK_B values calculated from dose-ratios (DR) at half maximal responses to CGP 12177A were determined for the antagonist concentration [*B*] from the equation, $pK_B = \log (DR-1) - \log [B]$, where DR is the dose ratio obtained at half maximal responses (Furchgott, 1972).

Non-linear regression was used to obtain pEC_{50} values from concentration-response curves. Individual points between sham and MI animals for each dose on the concentration-response curve were analysed using an unpaired Student's *t*-test. Differences between curves were analysed using repeated measures ANOVA (RM ANOVA), to obtain a *P* value.

Drugs used

Alfaxan (alphaxalone + alphadolone acetate) (Jurox, Silverwater, Australia), lignocaine hydrochloride (Delta West Pty Ltd, WA, Australia), (−)-CGP 12177A, phenoxybenzamine (Research Biochemicals International, Natick, MA, U.S.A.), CGP 20712A (Ciba-Geigy, Basel, Switzerland), ICI 118,551 (Zeneca, Wilmslow, Cheshire, U.K.), (−)-isoprenaline bitartrate, (−)-propranolol hydrochloride, pertussis toxin, reserpine (Sigma, St. Louis, MO, U.S.A.); (−)-RO 363 (Institute of Drug Technology, Boronia, Australia), (−)-bupranolol (Sanol, Monheim, Germany).

Results

Model of cardiac failure

A total of 91 rats were used in these studies, 31 rats underwent sham operations of which 100% survived, and 60 rats underwent coronary artery ligation (MI) of which 82%

Table 1 Tissue weight/body weight ratio (mg g^{-1}) and infarct size in sham and MI operated rats after 4-weeks ligation

	4-week treatment		
	Sham (n = 31)	*MI* (n = 33)	P *value*
Body weight (g)	*305.6* ± 3.3	302.1 ± 4.4	0.522
Tissue weight/body weight (mg g^{-1})			
Lung	3.83 ± 0.06	5.16 ± 0.13*	<0.001
Left ventricle	1.39 ± 0.03	1.25 ± 0.03†	0.004
Right ventricle	0.50 ± 0.01	0.65 ± 0.01*	<0.001
Left atrium	0.11 ± 0.01	0.21 ± 0.01*	<0.001
Infarct size			
Epicardial scar as a percentage of left ventricle	–	53.6 ± 1.3	
Endocardial scar as a percentage of left ventricle	–	52.5 ± 1.3	

Values are expressed as mean ± s.e.mean. *For significant increase compared with relative sham group. †For significant decrease compared with relative sham group.

survived (11 out of 60 animals died within 48 h), using the method of Gu *et al.* (1998). Of the 60 animals that underwent MI, 33 had an infarct size >45% of the LV and were used in the study, while 27 were excluded because of an infarct size >45% of the LV. Body weight pre- (sham 290.4±3.9 g ($n=31$), MI 291.4±4.4 g ($n=33$), $P=0.938$) and post-operatively (sham 305.6±3.3 g ($n=31$), MI 302.1±4.4 g ($n=33$), $P=0.522$) was not significantly different between sham-operated and MI rats.

Four weeks after surgery, organ and tissue weights were measured from sham-operated and MI rats and expressed as a proportion of body weight. Right ventricular weight (+30%), lung weight (+35%) and left atrial weight (+83%) were all significantly increased after MI compared to the sham group (Table 1), indicating congestion of the lungs and right ventricular hypertrophy representative of cardiac failure. Left ventricular weight was significantly decreased (−9.7%) mostly due to the replacement of healthy myocardium by scar tissue. Animals with MI included in this study had a minimum infarct size of 45% as determined by the epicardial left ventricular surface area (Gu *et al.*, 1998). We have previously shown that these morphological changes are associated with reduced systolic blood pressure and raised left ventricular end diastolic pressure (Kompa *et al.*, 1999).

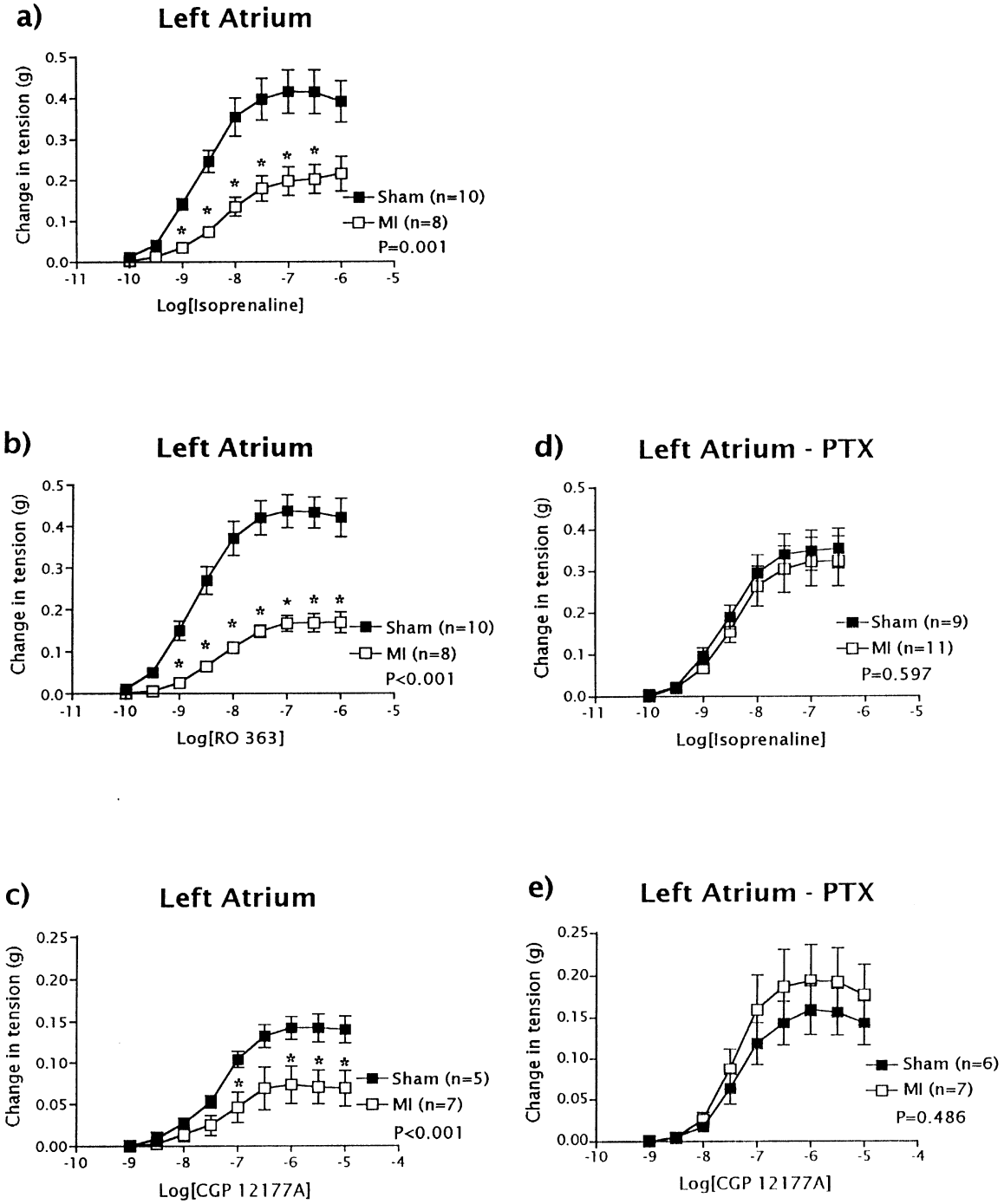

Figure 1 In electrically stimulated left atrium, myocardial infarction (MI) reduces the maximum inotropic responsiveness to isoprenaline (a), RO 363 (b) and CGP 12177A (c), *indicates $P<0.05$ for sham versus MI. The P value on each graph is determined from a RM ANOVA between the sham and MI curve. Pertussis toxin (PTX) treatment (10 $\mu g\ kg^{-1}$, i.p.) 3 days prior to experimentation restores this responsiveness to isoprenaline (d) and CGP 12177A (e) in MI-treated animals to similar levels seen in the sham group.

Comparison of β_1- and β_4-adrenoceptor responses in a rat model of cardiac failure

Inotropic responses to isoprenaline (sham 4.1±0.6 mN, $n=10$; MI 2.1±0.4 mN, $n=8$, $P<0.02$; −48%), RO 363 (sham 4.2±0.5 mN, $n=10$; MI 1.8±0.3 mN, $n=8$, $P<0.005$; −61%) and CGP 12177A (sham 1.4±0.1 mN, $n=5$; MI 0.7±0.2 mN, $n=7$, $P<0.05$; −49%) in field stimulated left atrium were significantly reduced in the MI group. Concentration-response curves to isoprenaline (pEC_{50}: sham 8.79±0.08, $n=10$; MI 8.30±0.10, $n=8$; $P=0.001$) and RO 363 (pEC_{50}: sham 8.69±0.07, $n=10$; MI 8.33±0.10, $n=8$; $P<0.01$) but not CGP 12177A (pEC_{50}: sham 7.42±0.10, $n=5$; MI 7.08±0.14, $n=7$; $P=0.089$) were significantly shifted to the right after MI (Figure 1a–c).

Chronotropic responses to isoprenaline (pEC_{50}: sham 8.7±0.07, $n=9$; MI 8.64±0.06, $n=8$; $P=0.535$), RO 363 (pEC_{50}: sham 8.87±0.08, $n=9$; MI 8.73±0.04, $n=8$; $P=0.167$) and CGP 12177A (pEC_{50}: sham 7.39±0.14, $n=5$; MI 7.38±0.10, $n=5$; $P=0.963$) in spontaneously beating right atrium (Figure 2a–c) were not significantly altered between sham and MI groups.

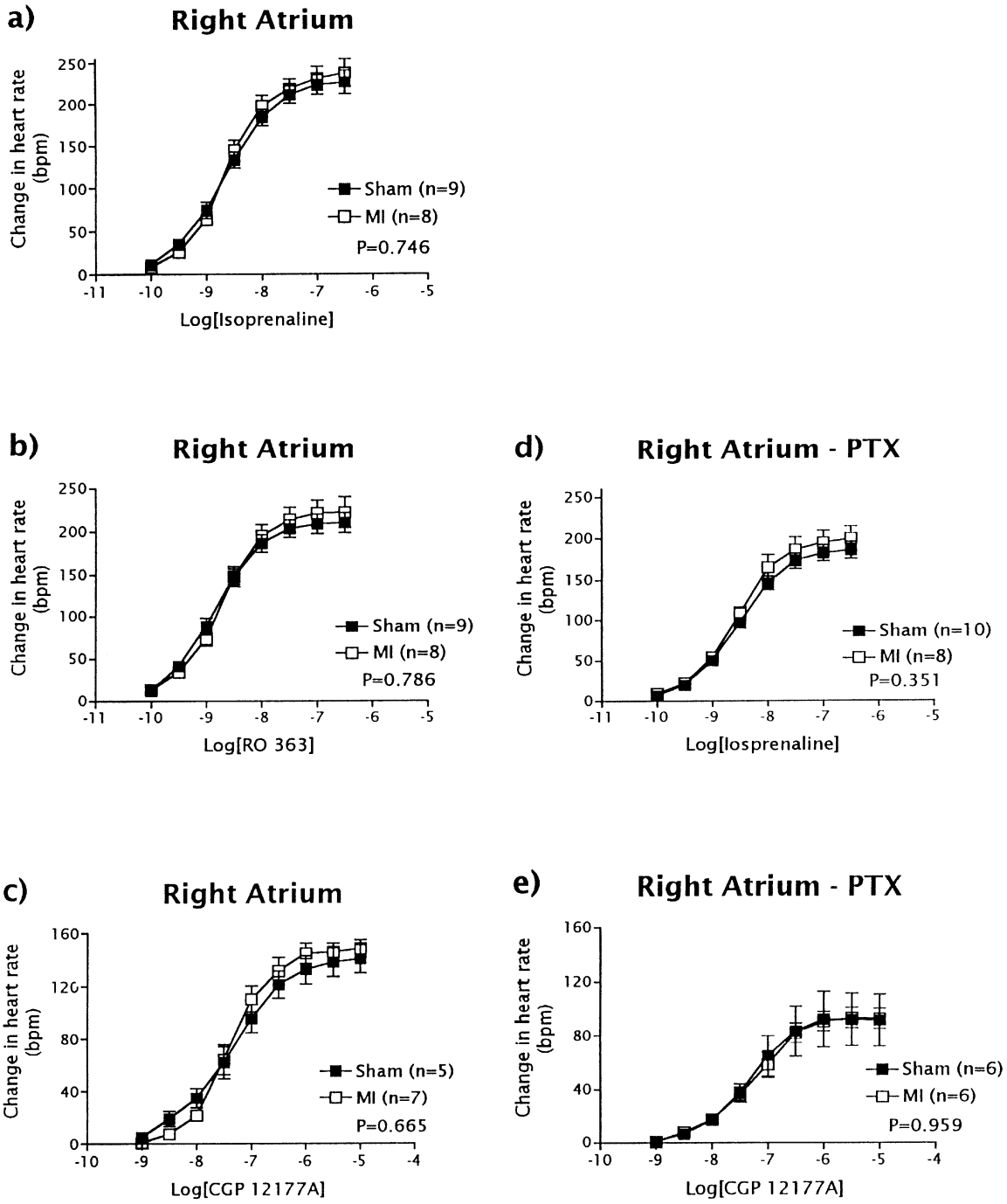

Figure 2 In spontaneously beating right atrium, myocardial infarction (MI) does not alter responsiveness to isoprenaline (a), RO 363 (b) and CGP 12177A (c). The P value on each graph is determined from a RM ANOVA between the sham and MI curve. Pertussis toxin (PTX) treatment (10 $\mu g\ kg^{-1}$, i.p.) 3 days prior to experimentation reduces the maximum chronotropic responsiveness to isoprenaline (d) and CGP 12177A (e) in both sham and MI-treated animals.

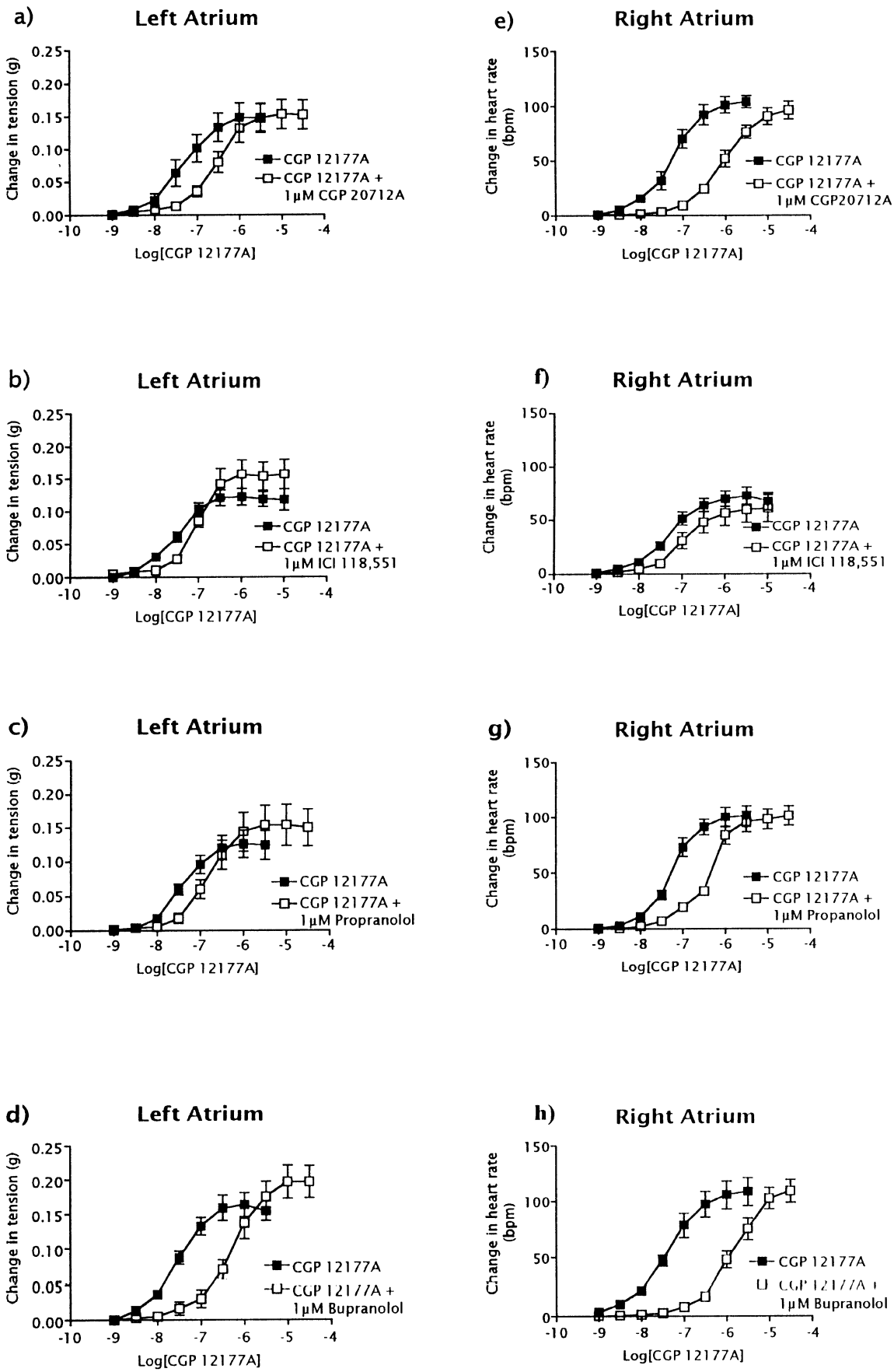

Figure 3 Concentration response curves to the putative β_4-adrenoceptor agonist CGP 12177A in left (a–d) and right (e–h) atria in the absence and presence of 1 μM of the following antagonists CGP 20712A (a,e), ICI 118,551 (b,f), propranolol (c,g) and bupranolol (d,h). pK_B values for each antagonist in left and right atria were calculated from 4–8 experiments and are expressed in Table 2.

Effect of pertussis toxin treatment on β_1- and β_4-adrenoceptor responses in a rat model of cardiac failure

We have recently shown that pertussis toxin (10 μg kg^{-1}, i.p., 3 days) pretreatment of rats with MI restores responsiveness to isoprenaline to levels indistinguishable from those observed in sham-operated animals (Kompa *et al*., 1999). In this study, as expected, pertussis toxin restored the maximum inotropic response and sensitivity to isoprenaline (pEC_{50}: sham 8.59 ± 0.08, $n=9$; MI 8.54 ± 0.05, $n=11$; $P=0.641$) but also restored the responses to CGP 12177A (pEC_{50}: sham 7.33 ± 0.09, $n=6$; MI 7.40 ± 0.06, $n=7$; $P=0.535$) to the same level as seen in the sham group (Figure 1d–e). Chronotropic responses in sham and MI animals to isoprenaline (pEC_{50}: sham 8.55 ± 0.04, $n=10$; MI 8.57 ± 0.04, $n=8$; $P=0.821$) and CGP 12177A (pEC_{50}: sham 7.36 ± 0.09, $n=6$; MI 7.25 ± 0.13, $n=6$; $P=0.512$) (Figure 2d,e) were unaffected after pertussis toxin.

pKB values for β-adrenoceptor antagonists at the putative β_4-adrenoceptor

pK_B values for the putative β_4-adrenoceptor were determined in right and left atria for various β-adrenoceptor antagonists: the β_1-adrenoceptor antagonist CGP20712A (1 μM); the β_2-adrenoceptor antagonist ICI 118,551 (1 μM); the β_1- and β_2-adrenoceptor antagonist propranolol (1 μM); and the non-selective β-adrenoceptor (β_1-, β_2- and putative β_4-adrenoceptor) antagonist bupranolol (1 μM). CGP 20712A, bupranolol, propranolol and ICI 118,551 showed moderate affinity for the putative β_4-adrenoceptor (Table 2; Figure 3).

Discussion

It has been postulated that a novel β-adrenoceptor found in heart and fat of several species, and yet to be cloned should be termed the 'putative β_4-adrenoceptor' (IUPHAR receptor code 2.1.ADR.B4.000.00.00.P; Bylund *et al*., 1998). Evidence for the existence of the putative β_4-adrenoceptor includes the observation that non-conventional partial agonists caused cardiostimulation at concentrations several orders of magnitude higher than those required to block β_1- and β_2-adrenoceptors in several species including cat, rat and guinea-pig (Kaumann, 1989; Kaumann & Molenaar, 1996). The inotropic response to CGP 12177A was also produced in human cardiac tissue (Kaumann, 1996; Molenaar *et al*., 1997).

Table 2 Affinity values (pK_B) for the β_1-, β_2- and putative β_4-adrenoceptor (AR)

Antagonist	*Affinity values*[a] *for* β_1-AR	β_2-AR	β_4-AR	*pK$_B$ left atria*[h]	*pK$_B$ right atria*[h]
CGP 20712A (β_1)	9.6[b]	5.4[b]	6.4[c]	6.7 ± 0.2	7.1 ± 0.1
ICI 118,551 (β_2)	7.2[d]	9.3[d]	<5.5[e]	6.4 ± 0.1	6.3 ± 0.1
Propranolol ($\beta_1+\beta_2$)	8.5[f]	8.9[f]	<5.7[e]	6.6 ± 0.1	6.8 ± 0.1
Bupranolol ($\beta_1+\beta_2+$'β_4')	9.1[g]	9.7[g]	7.3[c]	7.7 ± 0.1	7.2 ± 0.1

[a]Data represents pK_B values obtained from antagonist studies. [b]Lemoine & Kaumann (1991). [c]Kaumann & Molenaar (1997). [d]Bilski *et al*. (1983). [e]Kaumann & Molenaar (1996). [f]Gille *et al*. (1985). [g]Lemoine & Kaumann (1983). [h]Present study.

Bupranolol, a putative β_4-adrenoceptor antagonist (Kaumann & Molenaar, 1997), competitively blocks CGP 12177A induced lipolysis in rat and human fat cells, an effect which is not antagonized by the selective β_3-adrenoceptor antagonist SR 59,230A (Galitzky *et al*., 1997). In human fat cells, the lipolytic activity of CGP 12177A was also antagonized by increasing concentrations of the β_1-adrenoceptor antagonist CGP 20712A (Galitzky *et al*., 1997). These studies suggest that the putative β_4-adrenoceptor is different from the β_3-adrenoceptor, as concentrations of the β_3-adrenoceptor selective antagonist SR 59,230A failed to shift the concentration-response curve to CGP 12177A.

The putative β_4-adrenoceptor is clearly different from the β_3-adrenoceptor described in human heart which when stimulated produces a negative inotropic response (Gauthier *et al*., 1996). This study used human ventricular biopsies from cardiac transplant patients, all receiving immunosuppresive therapy with half the subjects on other drugs known to possess cardiovascular effects. These tissues were used to investigate the functional activity of β_3-adrenoceptor agonists BRL 37344, SR 58611, CL 316243 and CGP 12177A, which produced negative inotropic responses (Gauthier *et al*., 1996). Responses to BRL 37344 were inhibited after pertussis toxin treatment suggesting that this receptor is coupled to Gi. This study proposedly identified β_3-adrenoceptor mRNA in human ventricular tissue using polyA$^+$ RNA. However, the authors failed to mention the amount of polyA$^+$ RNA used in the PCR analysis and also failed to provide comparative PCR controls in other tissues known to contain β_3-adrenoceptor mRNA, such as adipose tissue, colon or ileum performed with the same amount of polyA$^+$ RNA and with defined conditions including cycle number. PolyA$^+$ RNA is predominantly mRNA and therefore represents approximately 20 times enrichment compared to total RNA. Using the same amount of polyA$^+$ RNA and total RNA in a PCR amplification, the polyA$^+$ RNA sample would be expected to yield a far greater β_3-adrenoceptor signal at 30 cycles than the total RNA sample. This is the only study to date to report these functional and molecular findings in human heart (Gauthier *et al*., 1996), and others have failed to repeat these functional experiments (Harding, 1997). The putative β_4-adrenoceptor also differs from the β_3-adrenoceptor in that it produces positive inotropic and chronotropic effects and couples to adenylate cylase to increase cyclic AMP presumably *via* Gs (Kaumann *et al*., 1997). It also increases the activity of cyclic AMP-dependent protein kinase, with these effects being potentiated by IBMX (Kaumann & Lynham, 1997). The putative β_4-adrenoceptor is therefore clearly distinct from the β_3-adrenoceptor in human heart. In addition, in β_3-adrenoceptor knock-out mice, the putative β_4-adrenoceptor response to CGP 12177A persists to produce inotropic and chronotropic responses similar to those observed in the wild type animals. Binding studies with [^{3}H]-CGP 12177A also showed similar B_{max} values in the two groups (Kaumann *et al*., 1998). Thus the putative β_4-adrenoceptor is not the β_3-adrenoceptor. In addition, in rat cardiac tissue, β_3-adrenoceptor mRNA is not found (Evans *et al*., 1996), indicating that the receptor is not expressed in rodent heart.

We have shown that CGP 12177A produces inotropic (40% of maximum response to isoprenaline) and chronotropic (60% of maximum response to isoprenaline) responses in rat atria, similar potencies to those shown in previous studies (Kaumann & Molenaar, 1996). In the rat model of cardiac failure we have shown that the maximum inotropic response to isoprenaline, RO 363 and CGP 12177A in left atria undergoes desensitization, and that pretreatment of the infarcted animals with

pertussis toxin restores the response to both isoprenaline and CGP 12177A back to control values. Thus desensitization and resensitization of the inotropic response to β_1- and putative β_4-adrenoceptors occurs in parallel.

We propose two possible explanations for these findings; firstly, both receptors may use the same signalling pathway. In healthy heart, β-adrenoceptors couple to adenylate cyclase *via* Gs increasing cyclic AMP levels. This in turn activates cyclic AMP dependent protein kinase which phosphorylates L-type calcium channels and phospholamban and leads to an increase in intracellular calcium which interacts with contractile proteins leading to a response. In this study we have shown that β-adrenergic responsiveness is reduced in a rat model of cardiac failure, and that the response is restored 3 days after administration of pertussis toxin. We have also shown that in this model, Giα_2 mRNA is increased (Kompa *et al*., 1999). This suggests that in heart failure, β-adrenoceptors can also couple to Gi as well as Gs, reducing β-adrenoceptor responsiveness. We suggest that the putative β_4-adrenoceptor, like the β_1-adrenoceptor in cardiac failure, can also couple to Gi to reduce inotropic responsiveness, and that pertussis toxin treatment, which inactivates Gi, blocks this pathway causing the receptors to couple solely to Gs and restore the response. Evidence exists to suggest that putative β_4-adrenoceptors produce their responses through a cyclic AMP dependent pathway, as shown by sensitization of the atrial response to isoprenaline and CGP 12177A by IBMX (Kaumann & Lynham, 1997). The second possibility is that responses and binding mediated by the putative β_4-adrenoceptor are in fact mediated by a low affinity site located on the β_1-adrenoceptor. Here we provide evidence in a model of cardiac failure that the β_1- and putative β_4-adrenoceptor undergo desensitization and resensitization to isoprenaline and CGP 12177A exactly in parallel. In addition, we showed that CGP 20712A and bupranolol interact with the putative β_4-adrenoceptor with approximately 100 times lower affinity values than that observed for the same antagonists blocking the β_1-adrenoceptor. To confirm that the β_1- and putative β_4-adrenoceptor are the same receptor, responses to CGP 12177A and other pindolol-like β-adrenoceptor antagonists need to be examined in β_1-adrenoceptor knockout mice (Rohrer *et al*., 1996).

Additional evidence that putative β_4-adrenoceptor responses may be produced *via* β_1-adrenoceptors comes from a study using hamster fibroblast (CHW) cells expressing the human β_1-adrenoceptor (hβ_1) (Pak & Fishman, 1996). In CHW-hβ_1 cells, CGP 12177A acted as an agonist with low potency, similar to that seen for putative β_4-adrenoceptor responses in heart (Kaumann & Molenaar, 1996, present study). The β_1-adrenoceptor antagonist, CGP 20712A (pA_2 7.6) was 100 times more potent than ICI 118,551 (pA_2 5.6) at inhibiting the cyclic AMP response to 20 nM CGP 12177A (Pak & Fishman, 1996). We determined the pA_2 values from the competition binding data (Figure 2, Pak & Fishman, 1996) using the definition that a pA_2 is the concentration of antagonist required to reduce the response by a dose ratio of two. Competition binding results from this study revealed that [^{3}H]CGP 12177A bound to two populations of binding sites in CHW-hβ_1 cells. Most binding sites (90%) displayed high affinity for CGP 12177A, the remaining 10% were of low affinity, indicating that the population of β_1-adrenoceptors was not homogenous. Like the putative β_4-adrenoceptor, which stimulates adenylate cyclase (Kaumann *et al*., 1997), CGP 12177A stimulated adenylate cyclase in membranes prepared from these CHW-hβ_1 cells (Pak & Fishman, 1996).

Using the rat MI model, we have shown that changes in desensitization with cardiac failure and resensitization (after pertussis toxin treatment) to isoprenaline and CGP 12177A occur in parallel, suggesting at least that the β_1- and putative β_4-adrenoceptors use the same signalling pathway. This is further supported by studies showing that the putative β_4-adrenoceptor uses the Gs/adenylate cyclase pathway and activates cyclic AMP dependent protein kinase (Kaumann *et al*., 1997; Kaumann & Lynham, 1997). We also showed that the β-adrenoceptor antagonists CGP 20712A, ICI 118,551, propranolol and bupranolol, which act at β_1-adrenoceptors, also interact with the putative β_4-adrenoceptor, identified by CGP 12177A, but with approximately 100 times lower affinity. This is in agreement with the Pak and Fishman (1996) study which showed that in CHW cells expressing the human β_1-adrenoceptor, CGP 20712A antagonized responses to CGP 12177A with an affinity 100 fold lower than at the β_1-adrenoceptor. Although this may suggest that the putative β_4-adrenoceptor is a novel subtype, when taken together with the fact that the putative β_4-adrenoceptor has yet to be cloned since its description several years ago, the results that we report here, and those of Pak & Fishman (1996), there is a strong case emerging that properties attributed to the putative β_4-adrenoceptor may be explained by an interaction of CGP 12177A at a low affinity state of the β_1-adrenoceptor.

This work was supported by the National Heart Foundation of Australia.

References

BENOVIC, J.L., SHORR, R.J., CARON, M.G. & LEFKOWITZ, R.J. (1984). The mammalian beta 2-adrenergic receptor: purification and characterization. *Biochemistry,* **23,** 4510 – 4518.

BILSKI, A.J., HALLIDAY, S.E., FITZGERALD, J.D. & WALE, J.L. (1983). The pharmacology of a β_2-selective adrenoceptor antagonist (ICI 118,551). *J. Cardiovasc. Pharmacol.,* **5,** 430 – 437.

BYLUND, D.B., BOND, R.A., CLARKE, D.E., EIKENBURG, D.C., HIEBLE, J.P., LANGER, S.Z., LEFKOWITZ, R.J., MINNEMAN, K.P., MOLINOFF, P.B., RUFFOLO, R.R., STROSBERG, A.D. & TRENDELENBURG, U.G. (1998). Adrenoceptors. In: Girdlestone, G. (ed). *The IUPHAR Compendium of Receptor Characterization and Classification*. IUPHAR Media:UK. pp. 58-74.

CARLSSON, E., ÅBLAD, B., BRÄNDSTRÖM, A. & CARLSSON, B. (1972). Differentiated blockade of the chronotropic effects of various adrenergic stimuli in the cat heart. *Life Sci.,* **11,** 953 – 958.

CARON, M.G., KOBILKA, B.K., FRIELLE, T., BOLANOWSKI, M.A., BENOVIC, J.L. & LEFKOWITZ, R.J. (1988). Cloning of the cDNA and genes for the hamster and human beta 2-adrenergic receptors. *J. Receptor Res.,* **8,** 7 – 21.

CUBERO, A. & MALBON, C.C. (1984). The fat cell beta-adrenergic receptor. Purification and characterization of a mammalian beta 1-adrenergic receptor. *J. Biol. Chem.,* **259,** 1344 – 1350.

EVANS, B.A., PAPAIOANNOU, M., BONAZZI, V.R. & SUMMERS, R.J. (1996). Expression of β_3-adrenoceptor mRNA in rat tissues. *Br. J. Pharmacol.,* **117,** 210 – 216.

FRIELLE, T., COLLINS, S., DANIEL, K.W., CARON, M.G., LEFKOWITZ R.J. & KOBILKA, B.K. (1987). Cloning of the cDNA for the human beta 1-adrenergic receptor. *Proc. Natl. Acad. Sci. U.S.A.,* **84,** 7920 – 7924.

FURCHGOTT, R.F. (1972). The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In: Blashko, H. & Muscholl, E. (eds.). *Handbook of Experimental Pharmacology*. Springer-Verlag: New York. pp. 283 – 335.

GALITZKY, J., LANGIN, D., VERWAERDE, P., MONTASTRUC, J.L., LAFONTAN, M. & BERLAN, M. (1997). Lipolytic effects of conventional β_3-adrenoceptor agonists and of CGP 12,177 in rat and human fat cells: preliminary pharmacological evidence for a putative β_4-adrenoceptor. *Br. J. Pharmacol.,* **122,** 1244–1250.

GAUTHIER, C., TAVERNIER, G., CHARPENTIER, F., LANGIN, D. & LEMAREC, H. (1996). Functional beta(3)-adrenoceptor in the human heart. *J. Clin. Invest.,* **98,** 556–562.

GILLE, E., LEMOINE, H., EHLE, B. & KAUMANN, A.J. (1985). The affinity of (−)-propranolol for β_1- and β_2-adrenoceptors of human heart. Differential antagonism of the positive inotropic effects and adenylate cyclase stimulation by (−)-noradrenaline and (−)-adrenaline. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **331,** 60–70.

GU, X.H., KOMPA, A.R. & SUMMERS R.J. (1998). Regulation of β-adrenoceptors in a rat model of cardiac failure: effect of perindopril. *J. Cardiovasc. Pharmacol.,* **32,** 66–74.

HARDING, S.E. (1997). Lack of evidence for β_3-adrenoceptor modulation of contractile function in human ventricular myocytes. *Circulation,* **96** (Suppl): I–53.

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

KAUFMANN, A.J. (1996). (−)-CGP 12177-induced increase of human atrial contraction through a putative third β-adrenoceptor. *Br. J. Pharmacol.,* **117,** 93–98.

KAUMANN, A.J. & LYNHAM, J.A. (1997). Stimulation of cyclic AMP-dependent protein kinase in rat atria by (−)-CGP 12177 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 β_3-adrenoceptors and is coupled to a cyclic AMP-dependent pathway in rat and human myocardium. *Br. J. Pharmacol.,* **120,** 102P.

KAUMANN, A.J. & MOLENAAR, P. (1996). Differences between the third cardiac β-adrenoceptor and the colonic β_3-adrenoceptor in the rat. *Br. J. Pharmacol.,* **118,** 2085–2098.

KAUMANN, A.J. & MOLENAAR, P. (1997). Modulation of human cardiac function through 4 β-adrenoceptor populations. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **355,** 667–681.

KAUMANN, A.J., PREITNER, F., SARSERO, D., MOLENAAR, P., REVELLI, J.P. & GIACOBINO, J.P. (1998). (−)-CGP 12177 causes cardiostimulation and binds to cardiac putative β_4-adrenoceptors in both wild-type and β_3-adrenoceptor knockout mice. *Mol. Pharmacol.,* **53,** 670–675.

KOMPA, A.R., GU, X.H., EVANS, B.A. & SUMMERS R.J. (1999). Desensitization of cardiac β-adrenoceptor signaling with heart failure produced by myocardial infarction in the rat. Evidence for the role of Gi but not Gs or phosphorylating proteins. *J. Mol. Cell Cardiol.,* **31,** 1185–1201.

LEMOINE, H. & KAUMANN, A.J. (1983). A model for the interaction of competitive antagonists with two receptor-subtypes characterized by a Schild-plot with apparent slope of unity. Agonist-dependent enantiomeric affinity ratios for bupranolol in tracheae but not in right atria of guinea-pigs. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **322,** 111–120.

LEMOINE, H. & KAUMANN, A.J. (1991). Regional differences of β_1- and β_2-adrenoceptor-mediated functions in feline heart. A β_2-adrenoceptor-mediated positive inotropic effect possibly unrelated to cyclic AMP. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **344,** 56–69.

MINNEMAN, K.P., HEDBERG, A. & MOLINOFF, P.B. (1979). Comparison of *beta* adrenergic receptor subtypes in mammalian tissues. *J. Pharmacol. Exp. Ther.,* **211,** 502–508.

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, the human cloned β_3-adrenoceptor and rodent intestinal β_3-adrenoceptors. *Br. J. Pharmacol.,* **120,** 165–176.

PAK, M.D. & FISHMAN, P.H. (1996). Anomalous behaviour of CGP 12177A on β_1-adrenergic receptors. *J. Recep. Signal Trans. Res.,* **16,** 1–23.

ROHRER, D.K., DESAI, K.H., JASPER, J.R., STEVENS, M.E., REGULA Jr. D.P., BARSH, G.S., BERNSTEIN, D. & KOBILKA, B.K. (1996). Targeted disruption of the mouse β_1-adrenergic receptor gene: Developmental and cardiovascular effects. *Proc. Natl. Acad. Sci. U.S.A.,* **93,** 7375–7380.

SARSERO, D., MOLENAAR, P. & KAUMANN, A.J. (1998). Validity of (−)-[^{3}H]-CGP 12177A as a radioligand for the 'putative β4-adrenoceptor' in rat atrium. *Br. J. Pharmacol.,* **123,** 371–380.

SUMMERS, R.J., STEPHENSON, J.A. & KUHAR, M.J. (1985). Localization of *beta* adrenoceptor subtypes in rat kidney by light microscopic autoradiography. *J. Pharmacol. Exp. Ther.,* **232,** 561–569.

(Received June 14, 1999
Revised August 27, 1999
Accepted September 3, 1999)