British Journal of Pharmacology (1996) 117, 93-98 © 1996 Stockton Press All rights reserved 0007-1188/96 $12.00

(−)-CGP 12177-induced increase of human atrial contraction through a putative third β-adrenoceptor

[1]Alberto J. Kaumann

Human Pharmacology Laboratory, The Babraham Institute, Cambridge CB2 4AT

1 The inotropic effects of (−)-4-(3-*t*-butylamino-2-hydroxypropoxy)benzimidazol-2-one ((−)-CGP 12177), an antagonist for β_1- and β_2-adrenoceptors as well as an agonist for β_3-adrenoceptors, were investigated on paced preparations of isolated right atrial appendages obtained from patients without advanced heart failure undergoing open heart surgery.

2 In the presence of (−)-propranolol (200 nM), (−)-CGP 12177 increased contractile force with a $-\log EC_{50}$, M, of 7.3. The maximum effects of (−)-CGP 12177 amounted to 15% and 11% of the effects of (−)-isoprenaline (400 μM) and of $CaCl_2$ (6.75 mM) respectively.

3 (−)-Bupranolol 1 μM, an antagonist with a pK_B of ~7.5 for β_3-adrenoceptors, antagonized surmountably the positive inotropic effects of (−)-CGP 12177 (in the presence of 200 nM (−)-propranolol) with an apparent pK_B of 7.3.

4 The potent positive inotropic effects of (−)-CGP 12177 and their resistance to blockade by (−)-propranolol but antagonism by (−)-bupranolol are consistent with the existence in human atrial myocardium of a minor third β-adrenoceptor population, possibly related to β_3-adrenoceptors.

Keywords: Human atrium; (−)-CGP 12177; positive inotropic effects; antagonism by (−)-bupranolol; β_3-adrenoceptors

Introduction

In mammalian heart the coexistence of a third β-adrenoceptor population, in addition to β_1- and β_2-adrenoceptors, has been suggested. The proposal was based on the properties of certain non-conventional partial agonists, the *in vitro* cardiostimulant potencies of which in isolated tissues of rat, guinea-pig and cat were considerably lower than their corresponding affinities for β_1- and β_2-adrenoceptors (Kaumann, 1973; 1989). In man, evidence for a third cardiac β-adrenoceptor population is elusive. The non-conventional partial agonist, pindolol, can cause tachycardia in man (Man In't Veld & Schalekamp, 1981) and it has been suggested that this may be due in part to activation of a third sinoatrial β-adrenoceptor population (Kaumann, 1989). However, Kaumann & Lobnig (1986) failed to detect positive inotropic effects of (−)-pindolol in human isolated atrium, presumably because the efficacy of (−)-pindolol for cardiac atypical β-adrenoceptors is low compared to other non-conventional partial agonists (Kaumann, 1983; Arch & Kaumann, 1993).

It is becoming increasingly clear that several non-conventional partial agonists have agonistic properties through native β_3-adrenoceptors (e.g. adipose tissue) (reviewed by Arch & Kaumann, 1993) as corroborated with cloned and transfected β_3-adrenoceptors (Emorine *et al.*, 1989). However, there is still uncertainty as to whether the third β-adrenoceptor proposed to function in mammalian heart is identical with the cloned β_3-adrenoceptor or is a distinct atypical β-adrenoceptor.

To address the question of the existence of a third β-adrenoceptor population in human heart the potent non-conventional partial agonist (−)-CGP 12177 was chosen as a tool because (i) it exhibits similar potencies as a cardiostimulant in several feline heart regions (Kaumann, 1983; 1989) and an agonist for native β_3-adrenoceptors (reviewed by Arch & Kaumann, 1993), including those of man (Lönnqvist *et al.*, 1993), as for cloned human β_3-adrenoceptors (Emorine *et al.*, 1989; Blin *et al.*, 1993), and (ii) its intrinsic activity for cardiac atypical β-adrenoceptors is larger than that of pindolol (Kaumann, 1983; see also review of Arch & Kaumann, 1993).

As expected for a non-conventional partial agonist (Kaumann, 1989), the affinity of (−)-CGP 12177 for the cloned human β_3-adrenoceptors is around two orders of magnitude lower than for human β_1- and β_2-adrenoceptors (Blin *et al.*, 1993). Both atypical β-adrenoceptors and β_3-adrenoceptors are resistant to blockade by (−)-propranolol at concentrations that saturate up to 99% of both β_1- and β_2-adrenoceptor populations (Harms *et al.*, 1977; additional evidence reviewed in Arch & Kaumann, 1993) but are blocked by (−)-bupranolol (Walter *et al.*, 1984; Kaumann, 1989; Langin *et al.*, 1991; Blin *et al.*, 1994). Experiments were therefore carried out in the absence and presence of (−)-propranolol alone and in combination with (−)-bupranolol.

Methods

Right atrial appendages were obtained from 11 patients undergoing surgery at Papworth Hospital, 10 for coronary artery bypass grafts and one for aortic valve replacement. Seven of the patients with coronary heart disease received β-adrenoceptor blockers for at least 3 months until and including the day of surgery. Sex, age and β-adrenoceptor blocker used were: 4 males (58, 60, 67 and 75 y atenolol), 2 males (58 and 60 y metoprolol) and one female (44 y atenolol). The patient with aortic valve replacement (male 64 y) and 3 male patients (56, 57 and 71 y) with coronary artery disease did not receive treatment with a β-adrenoceptor antagonist. Some of the patients had also been prescribed some of the following drugs: aspirin, isosorbide mononitrate, nifedipine, nicardipine, diltiazem, bezafibrate, enalapril, amiodarone, ranitidine and cimetidine. After excision, the appendages were immediately placed in modified oxygenated Krebs solution at room temperature containing (mM): Na^+ 125, K^+ 5, Ca^{2+} 2.25, Mg^{2+} 0.5, Cl^- 98.5, SO_4^{2-} 0.5, HCO_3^- 29, HPO_4^{2-} 1, EDTA 0.04 and equilibrated with 95% O_2/5% CO_2. The appendages were sectioned into 2 to 3 strips and set up to contract at 1 Hz in an apparatus with a 50 ml bath (Blinks, 1965) in the solution described above supplemented with (mM): Na^+ 15, fumarate 5, pyruvate 5, L-glutamate 5, glucose 10 at 37°C. The tissues were attached to Swema SG 4-45 strain gauge transducers and force

[1] Author for correspondence.

recorded on a Watanabe polygraph. The strips were driven with square-wave pulses of 5 ms duration and of just over threshold voltage. After the determination of a length-tension curve, the length of each strip was set to obtain 50% of the resting tension associated with maximum developed force.

A single cumulative concentration-effect curve to (−)-CGP 12177 was determined for each strip in the absence of (−)-propranolol, in the presence of (−)-propranolol 200 nM and/or in the presence of both (−)-propranolol 200 nM and (−)-bupranolol 1 μM. Antagonists were present for 45 min before a curve was begun. Whenever possible, depending on size of the atrial appendage, the three curves were obtained on strips dissected from the same atrial appendage. The experiments were concluded by the administration of a β-adrenoceptor saturating concentration of (−)-isoprenaline (400 μM) and after an equilibrium response to (−)-isoprenaline was established, by raising the Ca^{2+} concentration to 6.75 mM. To assess whether blockade by (−)-bupranolol was surmountable, the maximum effects of (−)-CGP 12177 in the absence and presence of (−)-bupranolol were computed as a percentage of the responses to both (−)-isoprenaline (400 μM) and 6.75 mM Ca^{2+}. The concentration-ratio of (−)-CGP 12177 in the presence and absence of 1 μM (−)-bupranolol was calculated at the EC_{50} levels using data obtained from two strips in the same atrial appendage. All results are expressed as mean ± s.e.mean.

The following drugs were gifts: (−)-CGP 12177 hydrochloride from Dr Lee Beeley (SmithKline Beecham, Welwyn, UK) and (−)-bupranolol from Dr Klaus Sandrock (Sanol, Monheim, Germany). (−)-Isoprenaline hydrochloride and (−)-propranolol hydrochloride were purchased from Sigma Chemical Co. (Poole, Dorset, UK).

Results

(−)-CGP 12177 6 nM depressed atrial force by 30 ± 7%; 20 nM did not cause further depression (Figure 1). There was a tendency for depression of atrial force to be reduced between 60 and 600 nM, but this effect was not significant. Further higher concentrations (2–6 μM) again caused depression (Figure 1). (−)-Propranolol (200 nM) depressed atrial force by 42 ± 8% (n = 11 strips)(not shown). The joint administration of (−)-propranolol 200 nM and (−)-bupranolol 1 μM depressed atrial force by 39 ± 7% (n = 8 strips) (not shown). The variable depression of contractile force induced by the 3 compounds is likely to be due to blockade of β_1-adrenoceptors, activated by small amounts of noradrenaline released from intra-atrial nerves with each pacing stimulus (Blinks, 1966).

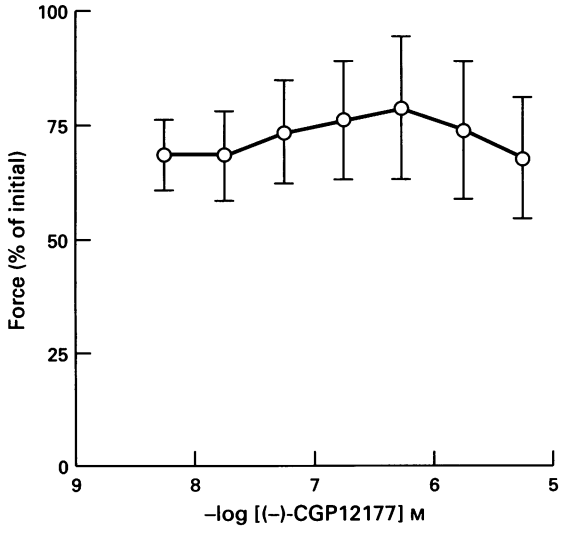

Figure 1 Concentration-effect curve to (−)-CGP 12177. Pooled results of 2 strips from 2 patients not treated with a β-adrenoceptor antagonist and 7 strips from 6 patients chronically treated with a β-adrenoceptor antagonist.

(−)-CGP 12177 caused small but significant concentration-dependent positive inotropic effects in the presence of 200 nM (−)-propranolol (Figures 2–4, Table 1). These effects reached equilibrium in approximately 5 min (Figures 2 and 3). A prolonged exposure to a single (−)-CGP 12177 concentration (100 nM) revealed slow fade of the positive inotropic response (Figure 2). Concentrations of (−)-CGP 12177 equal to and greater than 600 nM tended to depress contractile force (Figures 2–4), probably as a result of fade induced by lower concentrations administered cumulatively. The effects of (−)-CGP 12177 in the presence of 200 nM (−)-propranolol did not differ significantly on atria obtained from patients chronically treated or not treated with β-adrenoceptor antagonists (Figure 4, Table 1).

(−)-Bupranolol (1 μM) in the presence of 200 nM (−)-propranolol, antagonized the positive inotropic effects of (−)-CGP 12177 in a surmountable manner (Figures 3 and 4, Table 1). (−)-CGP 12177 20 μM did not depress contractile force in the presence of (−)-bupranolol (and (−)-propranolol) (Figures 3 and 4).

Discussion

The positive inotropic effects of (−)-CGP 12177 point for the first time to the existence of atypical β-adrenoceptors (plausibly β_3-adrenoceptors) in human heart. This assertion is based on the potency of (−)-CGP 12177, the resistance of these effects to blockade by (−)-propranolol and the antagonism of these effects by (−)-bupranolol, as will be discussed in detail. The possible relationship of human atrial atypical β-adrenoceptors to β_3-adrenoceptors, the question of fade and the apparent lack of influence of chronic treatment of patients with β-adrenoceptor blocking agents will also be examined.

The potency of (−)-CGP 12177

The lipolytic potency on human fat (−log EC_{50}, M, = 7.3–7.8 Lönnqvist *et al.*, 1993) and positive inotropic potency of (−)-CGP 12177 (in the presence of 200 nM (−)-propranolol) in human atrium (−log EC_{50}, M, = 7.3) are similar, suggesting, but not proving, mediation through the same receptors in the 2 tissues. In feline heart tissues the potentices of (±)-CGP 12177 as a cardiostimulant (−log EC_{50}, M, between parentheses) in right ventricular papillary muscle (7.3), left atrium (7.9) and sinoatrial node (7.8) (Kaumann, 1983; Arch & Kaumann, 1993) are also similar to those of (−)-CGP 12177 for human atrium, suggesting that the receptors involved are species homologues.

Resistance to blockade by (−)-propranolol

The marginal stimulant effects that appeared to be elicited by 60–600 nM (−)-CGP 12177 (Figure 1) could conceivably be the net result of stimulant effects, mediated through a third β-adrenoceptor population, and depressant effects due to a still progressing blockade of β_1-adrenoceptors activated by small amounts of noradrenaline released with each pacing stimulus. Equilibrium blockade of β_1-adrenoceptors with 200 nM (−)-propranolol would therefore be expected to abolish the stimulant effects of neuronally-released noradrenaline and reveal stimulant effects of (−)-CGP 12177 unopposed by its own blocking effects of β_1-adrenoceptors. The trend of a smaller maximum cardiodepression caused by (−)-CGP 12177 (30%) compared to that induced by (−)-propranolol (42%) is in line with the above explanation. As expected, the positive inotropic effects of (−)-CGP 12177 became more prominent in the presence of 200 nM (−)-propranolol. Furthermore, (−)-CGP

12177 appeared to be at least as potent in the presence as in the absence of (−)-propranolol, consistent with mediation through β_3-adrenoceptors.

While (−)-propranolol is slightly but significantly selective for β_2-adrenoceptors ($pK_B = 8.9$, $pK_i = 9.15$) compared to β_1-adrenoceptors ($pK_B = 8.5$, $pK_i = 8.2$) both in isolated human atrium (Gille *et al.*, 1985) and cloned and transfected human receptors (Blin *et al.*, 1993) it has considerably lower affinity for cloned and transfected human β_3-adrenoceptors ($pK_i = 6.8$, Blin *et al.*, 1993).

(−)-Propranolol is also a weak partial agonist in cloned and transfected human β_3-adrenoceptors with a $-\log EC_{50}$, M, = 5.8 for adenylyl cyclase stimulation (Blin *et al.*, 1993) which could presumably be related to the high receptor density (2×10^5 receptors per transfected cell). It is noteworthy in this context to recall that in feline left atrium (±)-propranolol (1 μM) has actually been shown to cause a small but conspicuous increase in contractile force when stimulated with pulses of barely threshold intensity (Blinks, 1967), perhaps suggesting that feline atrial myocardium has a higher density of atypical β-adrenoceptors than human atrium. Unlike the thin feline atrial tissues used by Blinks (1967), viable preparations of human right atrial appendage are thicker and have a highly variable and irregular anatomy which requires a pacing current that also releases sufficient amounts of noradrenaline to activate to a variable extent β_1-adrenoceptors. This difference between feline and human atrial preparations is a factor that precludes the visualisation of very small stimulant effects by some non-conventional partial agonists. From the reviewed evidence it becomes apparent that 200 nM (−)-propranolol, the concentration used here, saturates β_1- and β_2-adrenoceptors by 96% and 99% respectively, ruling out their involvement in the mediation of the effects of (−)-CGP 12177. The observation that the positive inotropic potency of (−)-CGP 12177 appears to be at least as high in the presence of (−)-propranolol as in its absence would be expected if the receptors were β_3-adrenoceptors, for which (−)-propranolol has low affinity.

Antagonism by (−)-bupranolol

(−)-Bupranolol blocked the effects of (−)-CGP 12177 with an apparent pK_B of 7.3, a value identical to its binding pK_i, similar to a pK_B of 7.7 for this ligand on cloned and transfected human β_3-adrenoceptors (Blin *et al.*, 1993; 1994) and similar to pK_B values for this ligand of 7.3–7.5 for rat adipose β_3-adrenoceptors (Langin *et al.*, 1991). The agreement of these constants is consistent with competition of (−)-CGP 12177 and (−)-bupranolol for a receptor that resembles the cloned β_3-adrenoceptor.

Are human atrial receptors for (−)-CGP 12177 β_3-adrenoceptors?

As discussed above, the potency of (−)-CGP 12177, the ineffectiveness of (−)-propranolol and the blockade by (−)-bupranolol taken together are quantitatively consistent with the existence of β_3-adrenoceptors in human atrium. In addition, estimates of the binding affinity of (−)-CGP 12177

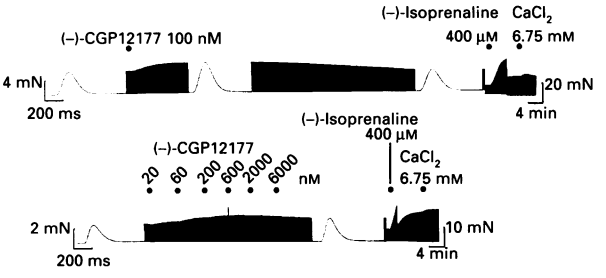

Figure 2 Time course of the inotropic effects of (−)-CGP 12177 observed in the presence of (−)-propranolol, 200 nM. Shown are original recordings obtained from 2 atrial strips of a 58 y male patient with coronary artery disease, chronically treated with (−)-atenolol. Top tracings: effects of 100 nM (−)-CGP 12177. Bottom tracings: cumulative concentration-effect curve to (−)-CGP 12177. The experiments were terminated by the administration of (−)-isoprenaline 400 μM followed by increasing the $CaCl_2$ concentration to 6.75 mM. Notice that the amplification was reduced before and again after the administration of (−)-isoprenaline by a final factor of 5.

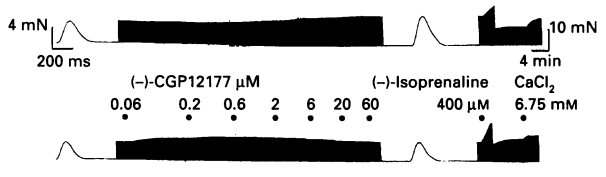

Figure 3 Surmountable antagonism by (−)-bupranolol of the positive inotropic effects of (−)-CGP 12177. Original recordings from 2 atrial strips of a 58 y male patient with coronary artery disease, chronically treated with metoprolol. Top tracing: strip incubated with both (−)-propranolol, 200 nM and (−)-bupranolol, 1 μM. Bottom tracing: strip incubated with (−)-propranolol 200 nM; calibrations as in top tracing. The dots refer to indicated drug additions to both strips. For further details see Figure 2.

Table 1 Comparison of the effects of (−)-CGP 12177 in atria from non-β-blocker-treated patients (non-βB) and patients treated with β-blockers (βB)

	Non-βB (n = 4)	*βB (n = 7)*
(−)-Propranolol (200 nM)	*5 strips*	*6 strips*
−log EC_{50} (M)	7.16 ± 0.17	7.46 ± 0.12
Maximum effect (% of (−)-isoprenaline 400 μM)	15.8 ± 3.9	13.7 ± 2.3
(−)-Propranolol (200 nM) plus (−)-bupranolol (1 μM)	*2 strips*	*6 strips*
−log EC_{50} (M)	5.56, 6.22	6.17 ± 0.07
Maximum effect (% of (−)-isoprenaline 400 μM)	28, 6.5	17.7 ± 1.7
log ((−)-CGP 12177 concentration-ratio) caused by (−)-bupranolol	1.82, 0.79	1.30 ± 0.05

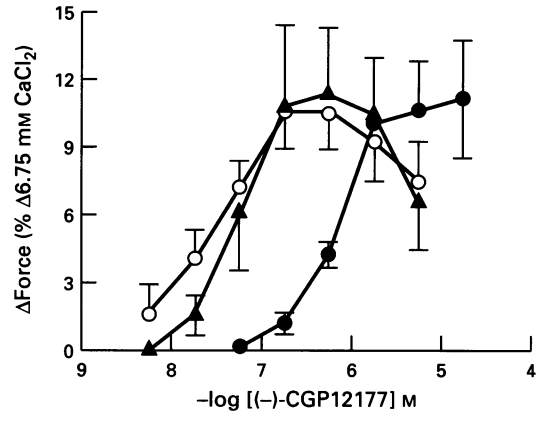

Figure 4 Cumulative concentration-effect curves to (−)-CGP 12177 obtained in the presence of (−)-propranolol 200 nM (▲, ○) and both (−)-propranolol 200 nM and (−)-bupranolol 1 μM (●). Shown are data from 5 strips from 4 patients not treated with a β-adrenoceptor antagonist (▲), 6 strips from 6 patients chronically treated with a β-adrenoceptor antagonist (○) and 8 strips (●, a pool of 2 strips from 2 patients not treated with a β-adrenoceptor antagonist and 6 strips from 6 patients chronically treated with a β-adrenoceptor antagonist).

($pK_i = 7.1$) for cloned human β_3-adrenoceptors (Blin *et al.*, 1993) as well as the binding affinity of [^{3}H]-CGP 12177 for β_3-adrenoceptors of human omental fat ($pK_D = 7.6$, Revelli *et al.*, 1993) also agree with the inotropic potency of (−)-CGP 12177 for human atrial receptors, suggesting that the latter are β_3-adrenoceptors. One would therefore expect to find mRNA for β_3-adrenoceptors in human atrium. However, although some authors have reported evidence for the location of mRNA for human β_3-adrenoceptors in a variety of tissues including gallbladder, colon, fat and neuroepithelioma cells (Krief *et al.*, 1993; Revelli *et al.*, 1993; Granneman *et al.*, 1993; Granneman & Lahners, 1994), Krief *et al.* (1993) also found mRNA for β_3-adrenoceptors in human heart, especially in left atrium, but always accompanied by mRNA for uncoupling protein, a marker of brown fat, and attributed the β_3-adrenoceptor mRNA as located in fat tissue surrounding the atria. However, Krief *et al.* (1993) did not formally exclude expression of β_3-adrenoceptor mRNA by other specialized cells; these cells could well be cardiomyocytes. Others failed to localize β_3-adrenoceptor mRNA in any human tissue, not even in fat (Thomas & Liggett, 1993), presumably because of technical differences with other authors. Thus, there is scope for further attempts to locate mRNA for the cloned β_3-adrenoceptors in human atrium and the present lack of this evidence of human atrial mRNA does not exclude the possibility that the positive inotropic effects of (−)-CGP 12177 are mediated through these receptors.

Three criteria have been proposed (Arch & Kaumann, 1993) which should be fulfilled for an effect to be considered as mediated through β_3-adrenoceptors. The effect should be (1) produced or mimicked by a non-conventional partial agonist, (2) resistant to blockade by blockers of β_1- and β_2-adrenoceptors and (3) produced or mimicked by agonists selective for β_3-adrenoceptors. Criteria (1) and (2) have been fulfilled but not (3) because the compound BRL 37344, selective for β_3-adrenoceptors especially in murine systems, causes a positive inotropic effect on human isolated atrium which is blocked by 100 nM (−)-propranolol with a potency consistent with mediation through β_2-adrenoceptors (preliminary experiments, Arch & Kaumann, 1993). A small residual effect of BRL 37344, mediated through human atrial atypical β-adrenoceptors, may have escaped attention. In this context, Wheeldon *et al.* (1994) have reported that the ester BRL 35135, which is completely demethylated to BRL 37344 *in vivo*, causes tachycardia in healthy volunteers that is resistant to blockade with the β_1-adrenoceptor-selective antagonist, bisoprolol but greatly reduced by nadolol (presumably through blockade of sinoatrial β_2-adrenoceptors). A very small but significant BRL 35135-evoked tachycardia (3 beats per min relative to predosing heart rate, coupled with a 4 beats per min fall in controls) was resistant to blockade by nadolol (Wheeldon *et al.*, 1994) and could be mediated through β_3-adrenoceptors because the affinity of nadolol for β_3-adrenoceptors is particularly low (Emorine *et al.*, 1989). Assuming that the nadolol-resistant marginal tachycardia reported by Wheeldon *et al.* (1994) is mediated through sinoatrial β_3-adrenoceptors, one would perhaps expect other full agonists for β_3-adrenoceptors to cause only minor increases in heart rate through these receptors.

It should be emphasised that even non-conventional partial agonists should have sufficient efficacy in order to allow the detection of significant stimulant effects. For example (−)-pindolol has a lower intrinsic activity (compared to (−)-isoprenaline) than (−)-CGP 12177 on both feline cardiac tissues (Kaumann & Blinks, 1980; Kaumann, 1983) and cloned human transfected β_3-adrenoceptors (Blin *et al.*, 1993) which may explain why it has not been possible to detect positive inotropic effects in human atrium with (−)-pindolol (Kaumann & Lobnig, 1986).

Fade of the (−)-CGP 12177 responses

The positive inotropic response to 100 nM (−)-CGP 12177 faded slowly and some depression of contractile force was also observed with 2–6 μM (−)-CGP 12177 during cumulative administration, suggesting desensitization. These experimental observations would appear to be inconsistent with an involvement of β_3-adrenoceptors which, unlike β_2-adrenoceptors that undergo desensitization through several mechanisms, were initially reported to be resistent to short-term desensitization (Granneman, 1992; Nantel *et al.*, 1993; Liggett *et al.*, 1993). Recent evidence is emerging, however, showing that β_3-adrenoceptors can desensitize following a 1 h (Chaudhry & Granneman, 1994) as well as a 24 h exposure to (−)-isoprenaline (Chambers *et al.*, 1994; Nantel *et al.*, 1995) as a function of cell type (Chaudhry & Granneman, 1994; Nantel *et al.*, 1995). Desensitization of β_3-adrenoceptors was assessed by a reduction of adenylyl cyclase stimulation (Chaudry & Granneman, 1994; Chambers *et al.*, 1994; Nantel *et al.*, 1995), a reduction of cyclic AMP-dependent protein kinase activity (Nantel *et al.*, 1995) and a reduction of Gs_α-protein levels (Chambers *et al.*, 1994). Directly relevant to the fade of the positive inotropic responses to (−)-CGP 12177 is the report of Chaudhry & Granneman (1994) that pretreatment of cells containing transfected human β_3-adrenoceptors with (−)-CGP 12177 also induces reduction of adenylyl cyclase stimulation mediated through these receptors. Therefore, the fade of the positive inotropic response of (−)-CGP 12177 is also consistent with mediation through β_3-adrenoceptors.

Lack of influence of chronic β-adrenoceptor blockade

The small number of tissues studied did not reveal a difference between the positive inotropic responsiveness to (−)-CGP 12177 in atria obtained from patients treated or not treated with the β_1-adrenoceptor-selective antagonists, atenolol and metoprolol. Work from the author's laboratory has previously shown that chronic treatment of patients with β-adrenoceptor blocking agents, usually selective for β_1-adrenoceptors, causes atrial inotropic hyperresponsiveness mediated through β_2-adrenoceptors (Kaumann *et al.*, 1989; Hall *et al.*, 1990), 5-HT_4 receptors (Sanders *et al.*, 1995) and histamine H_2 receptors (Kaumann *et al.*, 1995), as well as a higher incidence of experimental atrial arrhythmias mediated through both β_1- and β_2-adrenoceptors (Kaumann & Sanders, 1993), and 5-HT_4 receptors (Kaumann & Sanders, 1994). Because the positive inotropic responses to dibutyryl cyclic AMP in atrial (Hall *et al.*, 1990) and to forskolin in atrial myocytes (Sanders *et al.*, 1995) are not modified by chronic blockade of patients with β-

adrenoceptor blocking agents, it appears that intracellular cross-talk occurs between G_s protein-coupled receptors (i.e. β_1- and β_2-adrenoceptors, 5-HT_4 receptors and H_2 receptors) that affects their coupling to effectors including adenylyl cyclase. The atrial atypical β-adrenoceptors activated by (−)-CGP 12177 appear not to participate in this cross-talk between the other G_s protein-coupled receptors, which is modified by chronic blockade of β_1-adrenoceptors, but more evidence is necessary to substantiate this initial finding.

Conclusion

The positive inotropic effects of (−)-CGP 12177 are consistent with the existence of atypical β-adrenoceptors in human atrium. The argument that these receptors resemble the cloned β_3-adrenoceptor requires further experimental verification including the, as yet, unknown effects of the physiological agonist noradrenaline.

The author is grateful to the heart surgeons of Papworth Hospital for the supply of atrial tissues.

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(Received July 24, 1995
Revised August 23, 1995
Accepted September 19, 1995)