Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 458 (2003) 163–170
www.elsevier.com/locate/ejphar

The *indirect* negative inotropic effect of carbachol in β_1-adrenoceptor antagonist-treated human right atria

Thekla Wangemann[a], Christine Giessler[b], Pia Willmy-Matthes[b], Ralf-Edgar Silber[a], Otto-Erich Brodde[b,c,*]

[a] *Clinic for Cardio-Thoracic Surgery, Martin Luther University of Halle-Wittenberg, D-06097, Halle, Germany*
[b] *Institute of Pharmacology and Toxicology, Martin Luther University of Halle-Wittenberg, Halle, Germany*
[c] *University of Essen Medical School, D-45147, Essen, Germany*

Received 19 August 2002; received in revised form 6 November 2002; accepted 12 November 2002

Abstract

To find out whether *indirect* negative inotropic effects of carbachol (i.e. decreases in force of contraction that had been stimulated by cyclic AMP-increasing agents) might differ dependent on the agonist employed to increase contractile force in isolated human right atrium, we studied effects of carbachol on atria prestimulated with noradrenaline, terbutaline, histamine and serotonin. All four agonists increased right atrial adenylyl cyclase activity and contractile force, whereby increases for terbutaline, histamine and serotonin, but *not* for noradrenaline, were significantly larger in right atria from β_1-adrenoceptor antagonist-treated vs. non-β_1-adrenoceptor antagonist-treated patients. Carbachol (10^{-8}–10^{-3} M) concentration-dependently decreased agonist-stimulated contractile force: maximum decrease was not significantly different within the four agonists. pD_2 values for carbachol, however, were higher in atria from non-β_1-adrenoceptor antagonist-treated vs. β_1-adrenoceptor antagonist-treated patients.

We conclude that, in isolated human right atria, carbachol-induced *indirect* negative inotropic effect is not dependent from the agonist employed to increase (via cyclic AMP accumulation) contractile force. However, in atria from β_1-adrenoceptor antagonist-treated patients, carbachol-induced *indirect* negative inotropic effect is attenuated.

Keywords: Atrium, human, right; Muscarinic M_2 receptor; Carbachol; Negative inotropic effect, indirect; β_1-Adrenoceptor antagonist

1. Introduction

In human heart, rate and force of contraction are under dual control of sympathetic activation and parasympathetic inhibition. There is general agreement that, in the human heart, the predominant form of muscarinic receptors is the muscarinic M_2 receptor (for recent reviews, see Caulfield and Birdsall, 1998; Brodde and Michel, 1999; Dhein et al., 2001). Muscarinic M_2 receptors couple via a pertussis toxin sensitive G-protein ($G_{i/o}$) to inhibition of adenylyl cyclase and thereby cause ''indirect'' negative inotropic effects on human atrial and ventricular preparations when basal force of contraction had been elevated by cyclic AMP-increasing agents such as β-adrenoceptor agonists or forskolin. In addition, in human atrial (but not ventricular) myocardium stimulation of muscarinic M_2 receptors causes ''direct'' negative inotropic effects (i.e. decreases in basal force of contraction) through opening of an inwardly rectifying potassium channel via direct effects of the G-protein βγ-subunits (for review, see Yamada et al., 1998).

In the human heart there are several receptor systems that couple via G_s-protein to activation of adenylyl cyclase; the resulting increases in intracellular levels of cyclic AMP lead to increases in force of contraction. Among these receptors systems are β_1- and β_2-adrenoceptors, histamine H_2 receptors and 5-HT_4 receptors (for reviews, see Brodde, 1993; Brodde et al., 1995a; Kaumann and Molenaar, 1997). In the human heart, β_2-adrenoceptors are more effectively coupled to adenylyl cyclase than are β_1-adrenoceptors (for references, see Brodde and Michel, 1999). In addition, in right atria from patients chronically treated with β_1-adrenoceptor antagonists it has been shown that inotropic responses to

* Corresponding author. Departments of Pathophysiology and Nephrology, University of Essen Medical School, IG I, 9. OG, Hufelandstr. 55, Essen, D-45147, Germany. Tel.: +49-201-723-4467; fax: +49-201-723-5963.
E-mail address: otto-erich.brodde@uni-essen.de (O.E. Brodde).

0014-2999/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0014-2999(02)02763-2

β_2-adrenoceptor stimulation (Hall et al., 1990; Motomura et al., 1990), histamine H_2 receptor stimulation (Sanders et al., 1996) and 5-HT_4 receptor stimulation (Sanders et al., 1995) are sensitized. Thus, the aim of the present study was to find out whether, in isolated electrically driven human right atrial trabeculae (a) the *indirect* negative inotropic effect of carbachol might differ dependent on the agonist used to increase force of contraction via cyclic AMP-elevation, and (b) whether chronic β_1-adrenoceptor antagonist treatment of the patients might affect these *indirect* negative inotropic effects of carbachol. For this purpose we determined carbachol-effects on isolated atrial trabeculae from patients chronically treated with β_1-adrenoceptor antagonists or from non-β_1-adrenoceptor antagonist-treated patients with force of contraction increased by stimulation of β_1-adrenoceptors (by noradrenaline in the presence of phentolamine and the highly selective β_2-adrenoceptor antagonist ICI 118,551; Bilski et al., 1983), β_2-adrenoceptors (by terbutaline), histamine H_2 receptors (by histamine) and 5-HT_4 receptors (by serotonin).

2. Patients and methods

2.1. Patients

Right atrial appendages were obtained from 50 patients (38 male, 12 female) undergoing elective coronary artery bypass grafting without apparent heart failure (NYHA class I–II, $n=39$) or undergoing open heart surgery because of aortic valve disease ($n=9$, NYHA I–II except one patient NYHA II–III) or mitral valve insufficiency ($n=2$, NYHA III) after having given informed written consent. Patients were divided into two groups: one group of patients had been treated with β_1-adrenoceptor antagonists ($n=31$, predominantly metoprolol or bisoprolol, mean age 63 ± 2 years); the other group had *not* been treated with β-adrenoceptor antagonists for at least 6 weeks before operation ($n=19$, mean age 64 ± 2 years). Patients of both groups had received nitrates ($n=25$), calcium channel antagonists ($n=6$), angiotensin converting enzyme (ACE)-inhibitors ($n=23$), angiotensin I AT_1 receptor antagonists ($n=2$), diuretics ($n=16$), digitalis glycosides ($n=9$), heparine ($n=13$), hydroxymethyl glutaryl coenzyme A reductase inhibitors (statins) ($n=15$), acetyl salicylic acid ($n=5$) and antibiotics ($n=1$), alone or in combination.

Premedication usually consisted of 0.5 mg lorazepam given orally in the evening before and 0.5–1.0 mg given orally in the morning of surgery. The operation was done under balanced anesthesia consisting of midazolam, fentanyl and pancuronium bromide as muscle relaxant, as well as isoflurane (0.6–1.0% vol./vol.) for narcosis; N_2O was avoided in all cases. Controlled ventilation was performed with an inspired oxygen fraction of 50–100%. In all patients, right atrial appendages were removed during installation of cardiopulmonary bypass. Immediately after excision, all specimens were quickly frozen in liquid nitrogen (for adenylyl cyclase experiments) or directly used (for organ-bath experiments).

2.2. Organ-bath experiments

The preparation of the tissues was begun usually within 5–20 min of surgical removal in oxygenated Tyrode-solution at room temperature in order to minimize inadequate oxygenation. The right atrial appendages were dissected to yield trabecular strips (4–5 mm length and 1 mm or less in diameter) without endocardial damage. Usually two to three, sometimes four, trabecular strips were obtained from each right atrial appendage.

The preparations were mounted in 10 ml organ baths containing Tyrode-solution of the following composition (mM): NaCl 119.8; KCl 5.4; $CaCl_2$ 1.8; $MgSO_4$ 1.05; NaH_2PO_4 0.42; $NaHCO_3$ 22.6; glucose 5.05; EDTA 0.05; ascorbic acid 0.28; equilibrated with carbogen at 37 °C. Myocardial strips were electrically stimulated by square wave pulses of about 20% above threshold (3–12 V; mean: 8 V) at a frequency of stimulation of 1.0 Hz (Stimulator II, Hugo Sachs Elektronik, March-Hugstetten, Germany). The developed tension of the preparation (maintained under a resting tension of 4.9 mN) was recorded via a strain gauge on a Hellige recorder (Hellige, Freiburg, Germany). Preparations were allowed to equilibrate for at least 1 h in Tyrode-solution. Thereafter cumulative concentration–response curves for noradrenaline (in the presence of 55 nM ICI 118,551, 10 μM phentolamine and 1 μM desipramine-acting solely at β_1-adrenoceptors), terbutaline (in the presence of 300 nM of the highly selective β_1-adrenoceptor antagonist CGP 20712 A; Dooley et al., 1986—acting solely at β_2-adrenoceptors), histamine (in the presence of 300 nM CGP 20712 A—acting solely at histamine H_2 receptors), and serotonin (in the presence of 300 nM CGP 20712 A-acting solely at 5-HT_4 receptors) were determined as detailed elsewhere (Deighton et al., 1992; Zerkowski et al., 1993; Schäfers et al., 1994). Each muscle preparation was used for one concentration–response curve only to exclude desensitization phenomena.

When the concentration–response curves to the agonists had reached a plateau response, concentration–response curves for carbachol (10^{-8}–10^{-3} M) were determined as described elsewhere (Giessler et al., 1998); in order to correct for spontaneous decline in agonist-induced increase in force of contraction, one strip was always run without carbachol. Spontaneous decline in force of contraction over the period of a carbachol-concentration–response curve was $10.2 \pm 1.1\%$ ($n=43$).

2.3. Adenylyl cyclase determination

Adenylyl cyclase activity was assessed as described by Salomon et al. (1974) with minor modifications as detailed elsewhere (Brown et al., 1992). Membranes (35–45 μg of

protein) were incubated for 10 min at 30 °C in a final volume of 100 μl containing 40 mM HEPES buffer pH 7.4, 5 mM $MgCl_2$, 1 mM EDTA, 10 μM GTP, 500 μM ATP, approximately 1,000,000 cpm [α-^{32}P]ATP, 100 μM cyclic AMP and an ATP regenerating system (5 mM phosphocreatine and 50 units/ml creatine phosphokinase) in the presence or absence of isoprenaline (100 μM), noradrenaline (100 μM, in the presence of 55 nM ICI 118,551 and 10 μM phentolamine-acting solely at β_1-adrenoceptors), terbutaline (100 μM, in the presence of 300 nMCGP 20712 A-acting solely at β_2-adrenoceptors), histamine (100 μM, in the presence of 300 nMCGP 20712 A-acting solely at histamine H_2 receptors) and serotonin (100 μM, in the presence of 300 nMCGP 20712 A-acting solely at 5-HT_4 receptors). The reaction was stopped by addition of 100 μl buffer containing 50 mM Tris, 40 mM ATP, 1.4 mM cyclic AMP, 2% sodium dodecyl sulphate and [^{3}H]cyclic AMP (approximately 10,000 cpm) at pH 7.5; 800 μl water was then added.

The mixture was poured on to Dowex AG 50W-X4 anion-exchange columns (200–400 mesh, hydrogen form) and ATP was eluted with 2 × 1 ml water. The Dowex columns were then placed over neutral alumina columns and cyclic AMP was eluted from the Dowex with 4 ml water. The alumina columns were placed over scintillation vials and the cyclic AMP was eluted from the alumina columns with 5 ml 0.1 M imidazole (pH 7.3). Lumasafe plus scintillator (15 ml; Lumac-LSC, Groningen, The Netherlands) was added to the eluate and counted at 42% efficiency. The determined amount of [^{3}H]cyclic AMP in each vials was used to calculate the recovery of cyclic AMP for each column, and the amount of [^{32}P]cyclic AMP collected from each column was corrected for the recovery rate (usually 70–80%).

Protein content of the membranes was assessed by the method of Bradford (1976) using bovine immunoglobulin γ as standard.

2.4. Statistical evaluations

All values are means ± S.E.M. of *n* patients. Experimental data were fitted and analyzed by computer-supported iterative nonlinear regression analysis using the GraphPAD prism 3.0 program (GraphPAD software, San Diego, CA). Adenylyl cyclase activity is given as agonist-induced net increase in activity over basal activity in the presence of 10 μM GTP (Brodde et al., 1995b). Statistical significance of differences was analyzed by unpaired two-tailed Student's *t*-test; a *P*-value < 0.05 was considered to be significant. All statistical calculations were performed with the GraphPAD prism 3.0 program.

2.5. Drugs used

(±)-CGP 20712 A (1-[2-(3-carbamoyl-4-hydroxy)phenoxy)-ethyl-amino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl)phenoxy]-2 propanol methansulfonate) was kindly donated by Ciba-Geigy (Basel, Switzerland). (−)-Isoprenaline bitartrate, ICI 118,551 hydrochloride (erythro-(±)-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol), terbutaline sulfate, histamine dihydrochloride and carbachol (carbamylcholine chloride) were purchased from SIGMA (Deisenhofen, Germany), 5-hydroxytryptamine creatinine sulfate from SERVA (Heidelberg, Germany). [α-^{32}P]-ATP (specific activity, 30 Ci/mmol) and [^{3}H]-cyclic AMP (specific activity, 44.5 Ci/mmol) were obtained from New England Nuclear (Dreieich, Germany). All other chemicals were of the highest purity grade commercially available.

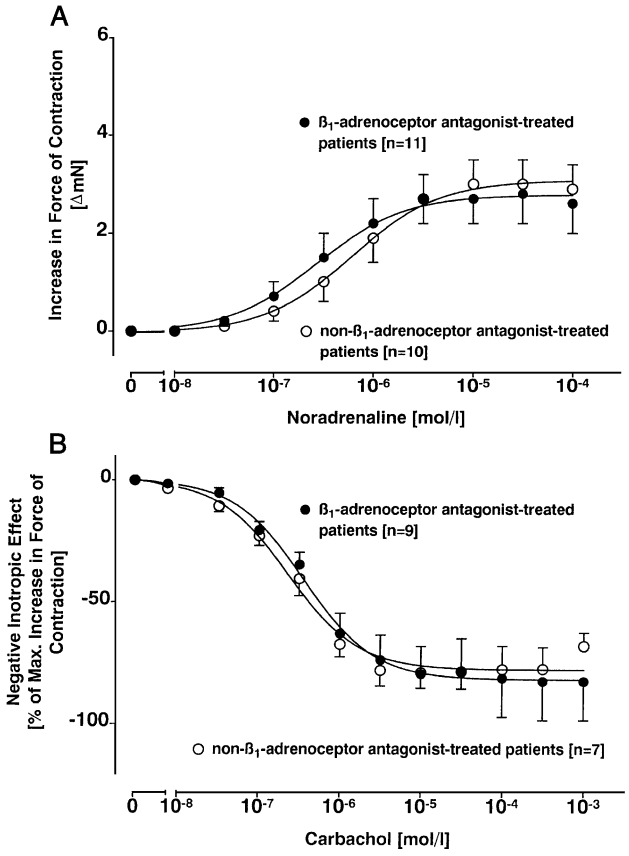

Fig. 1. (A) Positive inotropic effect of noradrenaline (in the presence of 55 nM ICI 118,551, 10 μM phentolamine and 1 μM desipramine) in isolated electrically driven human right atrial trabecular strips obtained from non-β_1-adrenoceptor antagonist-treated patients (open circles) and β_1-adrenoceptor antagonist-treated patients (closed circles). Ordinate: positive inotropic effect of noradrenaline in delta mN; Abscissa: molar concentrations of noradrenaline. Basal force of contraction was in non-β_1-adrenoceptor antagonist-treated patients 1.7 ± 0.3 mN and in β_1-adrenoceptor antagonist-treated patients 4.0 ± 0.4 mN ($P < 0.01$). (B) Negative inotropic effect of carbachol on isolated electrically driven human right atrial trabecular strips obtained from non-β_1-adrenoceptor antagonist-treated patients (open circles) and β_1-adrenoceptor antagonist-treated patients (closed circles) prestimulated with 10^{-4} M noradrenaline. Ordinate: negative inotropic effect of carbachol in % of maximal force of contraction (i.e. force of contraction enhanced by 10^{-4} M noradrenaline). Abscissa: molar concentrations of carbachol. Means ± S.E.M.; number of experiments (= number of patients studied) in parentheses.

3. Results

3.1. Positive inotropic effects

In right atrial trabecular strips obtained from non-β_1-adrenoceptor antagonist-treated patients, noradrenaline, terbutaline, histamine and serotonin concentration-dependently increased force of contraction; noradrenaline-, terbutaline- and histamine-induced maximal increases in force of contraction were comparable, while that evoked by serotonin was significantly lower (Figs. 1A–4A); pD_2 values for the four agonists are given in Table 1.

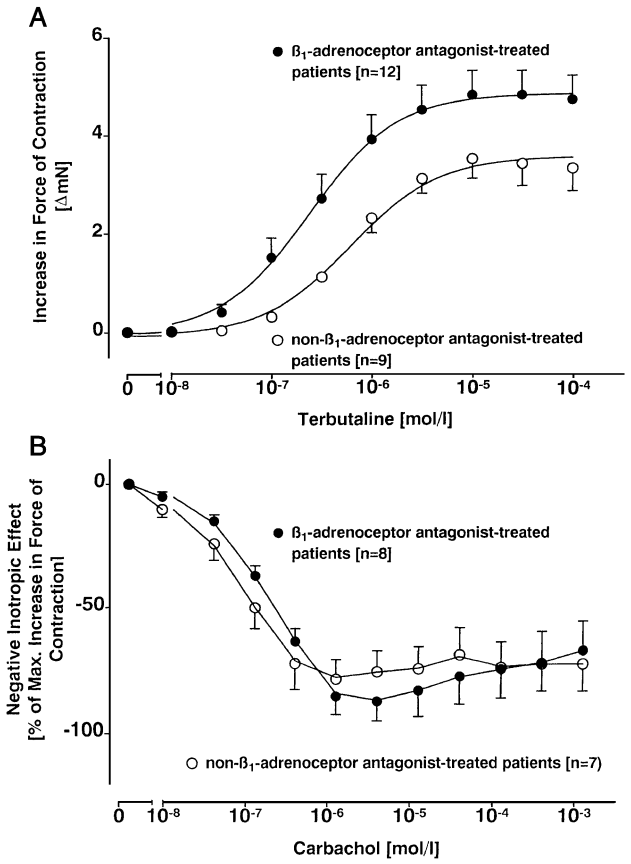

Fig. 2. (A) Positive inotropic effect of terbutaline (in the presence of 300 nM CGP 20712 A) in isolated electrically driven human right atrial trabecular strips obtained from non-β_1-adrenoceptor antagonist-treated patients (open circles) and β_1-adrenoceptor antagonist-treated patients (closed circles). Ordinate: positive inotropic effect of terbutaline in delta mN; Abscissa: molar concentrations of terbutaline. Basal force of contraction was in non-β_1-adrenoceptor antagonist treated-patients 1.3 ± 0.4 mN and in β_1-adrenoceptor antagonist-treated patients 2.7 ± 0.5 mN ($P < 0.05$). (B) Negative inotropic effect of carbachol on isolated electrically driven human right atrial trabecular strips obtained from non-β_1-adrenoceptor antagonist-treated patients (open circles) and β_1-adrenoceptor antagonist-treated patients (closed circles) prestimulated with 10^{-4} M terbutaline. Ordinate: negative inotropic effect of carbachol in % of maximal force of contraction (i.e. force of contraction enhanced by 10^{-4} M terbutaline). Abscissa: molar concentrations of carbachol. Means ± S.E.M.; number of experiments (= number of patients studied) in parentheses.

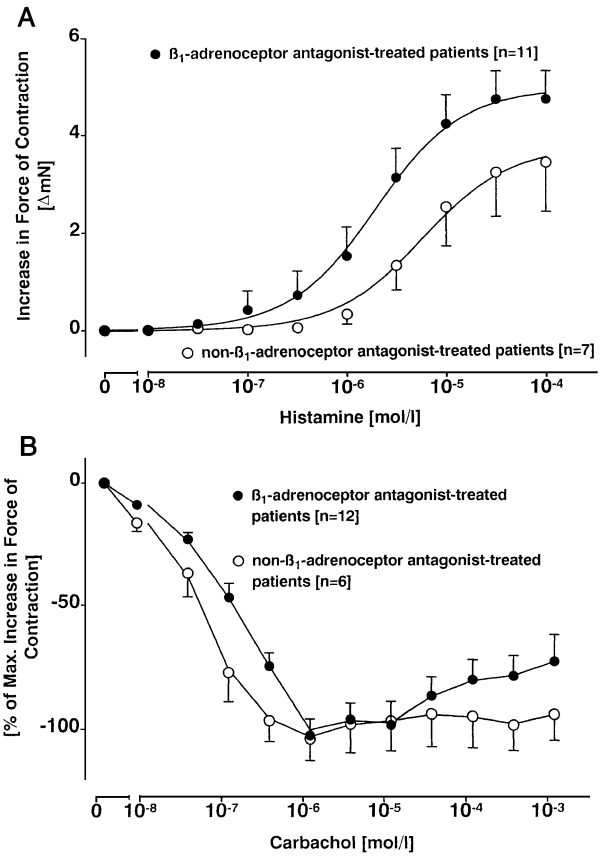

Fig. 3. (A) Positive inotropic effect of histamine (in the presence of 300 nM CGP 20712 A) in isolated electrically driven human right atrial trabecular strips obtained from non-β_1-adrenoceptor antagonist-treated patients (open circles) and β_1-adrenoceptor antagonist-treated patients (closed circles). Ordinate: positive inotropic effect of histamine in delta mN; Abscissa: molar concentrations of histamine. Basal force of contraction was in non-β_1-adrenoceptor antagonist-treated patients 1.2 ± 0.2 mN and in β_1-adrenoceptor antagonist-treated patients 2.4 ± 0.5 mN ($P < 0.05$). (B) Negative inotropic effect of carbachol on isolated electrically driven human right atrial trabecular strips obtained from non-β_1-adrenoceptor antagonist-treated patients (open circles) and β_1-adrenoceptor antagonist-treated patients (closed circles) prestimulated with 10^{-4} M histamine. Ordinate: negative inotropic effect of carbachol in % of maximal force of contraction (i.e. force of contraction enhanced by 10^{-4} M histamine). Abscissa: molar concentrations of carbachol. Means ± S.E.M.; number of experiments (= number of patients studied) in parentheses.

In right atrial trabecular strips obtained from patients preteated with β_1-adrenoceptor antagonists, in general basal force of contraction was higher than in trabecular strips from non-β_1-adrenoceptor antagonist-treated patients (see legends to Figs. 1A–4A). In these preparations concentration–response curve for increases in force of contraction induced by noradrenaline was superimposable to that obtained in atrial trabeculae from non-β_1-adrenoceptor antagonist-treated patients (Fig. 1A). In contrast, increases in force of contraction induced by terbutaline, histamine and serotonin were in preparations from patients treated with β_1-adrenoceptor antagonists significantly larger than in preparations from non-β_1-adrenoceptor antagonist-treated patients (Figs. 2A–4A).

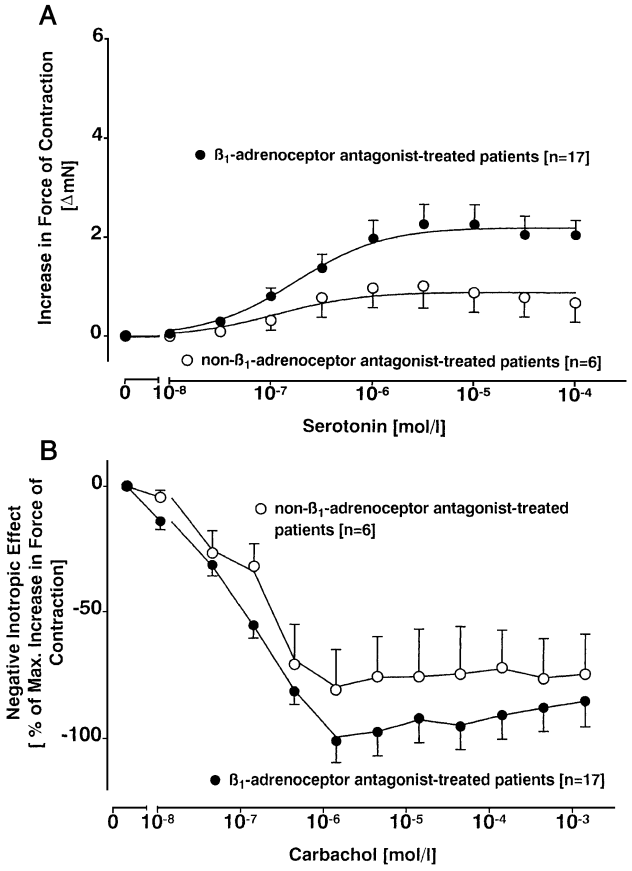

Fig. 4. (A) Positive inotropic effect of serotonin (in the presence of 300 nM CGP 20712 A) in isolated electrically driven human right atrial trabecular strips obtained from non-β_1-adrenoceptor antagonist-treated patients (open circles) and β_1-adrenoceptor antagonist-treated patients (closed circles). Ordinate: positive inotropic effect of serotonin in delta mN; Abscissa: molar concentrations of serotonin. Basal force of contraction was in non-β_1-adrenoceptor antagonist-treated patients 1.6 ± 0.5 mN and in β_1-adrenoceptor antagonist-treated patients 1.8 ± 0.3 mN. (B) Negative inotropic effect of carbachol on isolated electrically driven human right atrial trabecular strips obtained from non-β_1-adrenoceptor antagonist-treated patients (open circles) and β_1-adrenoceptor antagonist-treated patients (closed circles) prestimulated with 10^{-4} M serotonin. Ordinate: negative inotropic effect of carbachol in % of maximal force of contraction (i.e. force of contraction enhanced by 10^{-4} M serotonin). Abscissa: molar concentrations of carbachol. Means ± S.E.M.; number of experiments (= number of patients studied) in parentheses.

3.2. Indirect negative inotropic effects of carbachol

In right atrial trabecular strips with force of contraction enhanced by 100 μM of noradrenaline, terbutaline, histamine and serotonin, carbachol (10^{-8}–10^{-3} M) concentration-dependently decreased force of contraction (Figs. 1B–4B); maximum decrease in force of contraction was not significantly different within the four agonists. Moreover, maximal decrease in force of contraction induced by carbachol was not significantly different in preparations from patients treated with β_1-adrenoceptor antagonists and from non-β_1-adrenoceptor antagonist-treated patients (Figs. 1B–4B). However, pD_2 values for carbachol were higher in preparations from non-β_1-adrenoceptor antagonist-treated patients than in preparations from β_1-adrenoceptor antagonist-treated patients; this difference reached statistical significance for preparations precontracted with 100 μM terbutaline and histamine (Table 2).

Table 1
pD_2 values for agonist-induced positive inotropic effects in isolated electrically driven human right atrial trabecular strips

Agonists	β_1-adrenoceptor antagonist treated-patients pD_2 values	Non-β_1-adrenoceptor antagonist-treated-patients pD_2 values
Noradrenaline	6.51 ± 0.05 (11)	6.22 ± 0.05 (10)[a]
Terbutaline	6.60 ± 0.03 (12)	6.21 ± 0.07 (9)[a]
Histamine	5.71 ± 0.03 (11)	5.22 ± 0.06 (7)[a]
Serotonin	6.76 ± 0.07 (17)	6.90 ± 0.21 (6)

Means ± S.E.M.; number of experiments (= number of patients studied) in parentheses.
[a] $P < 0.05$ vs. corresponding values in β_1-adrenoceptor antagonist-treated patients.

3.3. Adenylyl cyclase activation

In a final set of experiments, we determined activation of adenylyl cyclase by the four agonists; for these experiments 100 μM of each agonist was used because we had previously shown that this concentration causes maximal activation of human right atrial adenylyl cyclase by noradrenaline (Brodde et al., 1984), terbutaline (Schäfers et al., 1994), histamine and serotonin (Zerkowski et al., 1993); for comparison effects of a saturating concentration of isoprenaline (100 μM) were assessed in the same preparations. Isoprenaline caused a net increase of adenylyl cyclase activity of 30.5 ± 3.9 pmol cyclic AMP/mg protein/min ($n = 10$) in atrial membranes from non-β_1-adrenoceptor antagonist-treated patients; this was slightly, but not significantly increased in atrial membranes from β_1-adrenoceptor antagonist-treated patients (36.9 ± 3.7 pmol cyclic AMP); similar data were obtained for noradrenaline-induced activation of adenylyl cyclase: net increase in activity was in atrial membranes from β_1-adrenoceptor antagonist-treated patients slightly higher than in membranes from non-β_1-adrenoceptor antagonist-treated patients. On the other hand,

Table 2
pD_2 values for carbachol-induced negative inotropic effects in isolated electrically driven human right atrial trabecular strips prestimulated with several agonists

Agonist used to stimulate atrial trabecular strips	β_1-Adrenoceptor antagonist-treated patients pD_2 values	Non-β_1-adrenoceptor antagonist-treated patients pD_2 values
Noradrenaline	6.45 ± 0.03 (9)	6.64 ± 0.08 (7)
Terbutaline	6.99 ± 0.09 (8)	7.28 ± 0.05 (7)[a]
Histamine	6.97 ± 0.08 (12)	7.39 ± 0.05 (6)[a]
Serotonin	7.21 ± 0.07 (17)	7.08 ± 0.09 (6)

Means ± S.E.M.; number of experiments (= number of patients studied) in parentheses.
[a] $P < 0.05$ vs. corresponding values in β_1-adrenoceptor antagonist-treated patients.

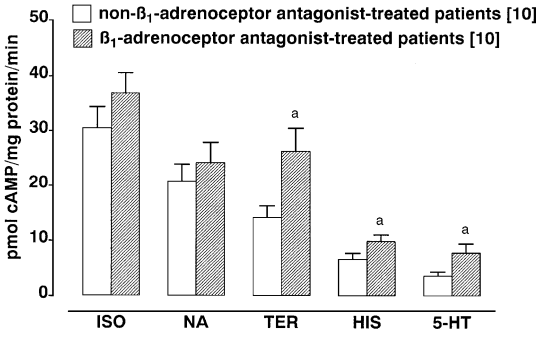

Fig. 5. Activation of adenylyl cyclase activity in right atrial membranes obtained from non-β_1-adrenoceptor antagonist-treated patients (open columns) and β_1-adrenoceptor antagonist-treated patients (hatched columns) prestimulated by 100 μM of isoprenaline (ISO), noradrenaline (NA, in the presence of 55 nM ICI 118,551 and 10 μM phentolamine), terbutaline (TER, in the presence of 300 nM CGP 20712 A), histamine (HIS, in the presence of 300 nM CGP 20712 A), and serotonin (5-HT, in the presence of 300 nM CGP 20712 A). Ordinate: net increase in adenylyl cyclase activity upon agonist-induced stimulation in pmol cyclic AMP formed/mg protein/min. Means ± S.E.M.; number of experiments (=number of patients studied) in parentheses. (a) $P<0.05$ vs. non-β_1-adrenoceptor antagonist-treated patients.

terbutaline-, histamine- and serotonin-induced activation of adenylyl cyclase was in atrial membranes from β_1-adrenoceptor antagonist treated patients significantly higher than in membranes from non-β_1-adrenoceptor antagonist-treated patients (Fig. 5).

4. Discussion

The main finding of the present study was that, in isolated electrically driven human right atria, (1) carbachol-induced *indirect* negative inotropic effect is not considerably dependent from the agonist to increase (via cyclic AMP accumulation) force of contraction and (2) carbachol-induced *indirect* negative inotropic effect appears, however, to be attenuated in atria obtained from β_1-adrenoceptor antagonist-treated patients.

In the present study noradrenaline, terbutaline, histamine and serotonin increased right atrial adenylyl cyclase activity and right atrial force of contraction. In agreement with previously published data from our group (Zerkowski et al., 1993, Schäfers et al., 1994) and from others (Baumann et al., 1983; Kaumann et al., 1989), noradrenaline (in the presence of phentolamine and ICI 118,551 acting solely at β_1-adrenoceptors), terbutaline (in the presence of CGP 20712 A acting solely at β_2-adrenoceptors) and histamine (acting at histamine H_2 receptors) caused approximately the same maximal increase in right atrial force of contraction, whereas serotonin (acting at 5-HT_4 receptors)-induced maximal inotropic effects were considerably lower (Kaumann et al., 1991; Zerkowski et al., 1993).

We (Michel et al., 1988; Motomura et al., 1990) and others (Hall et al., 1990) have previously shown that long-term treatment of patients with coronary artery disease with β_1-adrenoceptor antagonists increases right atrial β_1-adrenoceptor density and sensitizes right atrial β_2-adrenoceptor-, but *not* β_1-adrenoceptor-mediated increases in force of contraction. Thus, in right atria obtained from patients chronically treated with β_1-adrenoceptor antagonists the positive inotropic effects of the β_2-adrenoceptor partial agonists procaterol (Motomura et al., 1990) and salbutamol (Hall et al., 1990) were significantly enhanced, whereas those of noradrenaline were not significantly affected. Similar sensitizing effects of long-term β_1-adrenoceptor treatment have been also observed for right atrial histamine H_2 (Sanders et al., 1996) and 5-HT_4 receptors (Sanders et al., 1995). The data of the present study confirm these findings: in right atria from patients chronically treated with β_1-adrenoceptor antagonists, maximal increase in force of contraction induced by terbutaline, histamine and serotonin was significantly increased whereas that for noradrenaline was not.

The mechanism underlying this "cross-talk" between β_1-adrenoceptors and several other G_s-coupled receptors in the human right atrium is, however, not completely understood. One possibility could be an increase in right atrial G_s- or a decrease in right atrial G_i-protein following long-term β_1-adrenoceptor antagonist treatment. However, several studies have failed to find any differences in mRNA or protein levels of G_s and G_i in right atria from β_1-adrenoceptor antagonist treated vs. non-β_1-adrenoceptor antagonist-treated patients (Brodde et al., 1991; Ferro et al., 1993; Jia et al., 1995). Moreover, dibutyryl cyclic AMP (Hall et al., 1990) and forskolin (Sanders et al., 1995) caused nearly identical increases in force of contraction in isolated electricaly driven right atrial trabeculae from β_1-adrenoceptor antagonist treated and non-β_1-adrenoceptor antagonist-treated patients thus excluding the possibility that responsiveness to cyclic AMP might be altered. It has been suggested, therefore, that, in human right atrium, chronic β_1-adrenoceptor antagonist treatment might enhance coupling of β_2-adrenoceptors to G_s-protein. The fact that two other G_s-coupled receptors, the histamine H_2 and the 5-HT_4 receptor (Sanders et al., 1995, 1996, present data), are sensitized in atria from β_1-adrenoceptor antagonist treated patients supports this hypothesis. The final experimental proof of this hypothesis, however, is still lacking.

It is well known that, in the human heart, the predominant muscarinic receptor subtype is the muscarinic M_2 receptor that couples via a pertussis toxin sensitive G-protein ($G_{i/o}$) to inhibition of adenylyl cyclase and thereby causes "indirect" negative inotropic effects on human atrial and ventricular preparations when basal force of contraction had been elevated by cyclic AMP-increasing agents. This had been clearly demonstrated on β-adrenoceptor agonists or forskolin-prestimulated human right atrial and ventricular preparations (for reviews, see Brodde and Michel, 1999; Dhein et al., 2001). The present results confirm and extent these observations. They show that carbachol causes not

only *indirect* negative inotropic effects on atria that had been stimulated with β-adrenoceptor agonists (noradrenaline and terbutaline in this study) or forskolin, but also on atrial preparations where force of contraction had been increased by stimulation of histamine H_2 or 5-HT_4 receptors. Moreover, the extent of the *indirect* negative inotropic effect of carbachol did not differ significantly on atria stimulated either by β_1- or β_2-adrenoceptor, histamine H_2 receptor or 5-HT_4 receptor stimulation. From these results, it can be concluded that in human right atrial preparations, the *indirect* negative inotropic effect of carbachol is not considerably dependent on the agent that increases (via cyclic AMP accumulation) force of contraction. It should be mentioned, however, that in human right atria, carbachol causes also a *direct* negative inotropic effect (see Introduction). We have previously shown, in isolated electrically driven human right atria, that the pD_2 values for the *direct* and *indirect* negative inotropic effect of carbachol did not differ considerably (Deighton et al., 1990); thus, we believe that in the present study the estimated pD_2 values for carbachol reflect pD_2 values for the *indirect* negative inotropic effect.

In animal studies it has been shown that chronic treatment with β_1-adrenoceptor antagonists leads to a decrease in the number of cardiac muscarinic M_2 receptors (Marquetant et al., 1992; Borst et al., 1997). We have previously shown that muscarinic M_2 receptor density was also decreased on right atria from patients with coronary artery disease without apparent heart failure who were chronically treated with β_1-adrenoceptor antagonists (Motomura et al., 1990; Brodde et al., 1991). Moreover, we had found that the decrease in muscarinic M_2 receptor density was accompanied by a significant rightward shift of the concentration–response curve for the *indirect* negative inotropic effect of carbachol. The present results confirm these findings: in noradrenaline-, terbutaline- and histamine-prestimulated right atria pD_2 values for the *indirect* negative inotropic effect of carbachol were lower in β_1-adrenoceptor antagonist-treated patients than they were in non-β_1-adrenoceptor antagonist-treated patients. It might be possible that the lower sensitivity of carbachol in atria from β_1-adrenoceptor antagonist-treated patients is due to the fact that in these atria terbutaline and histamine caused lager increases in force of contraction than in atria from non-β_1-adrenoceptor antagonist-treated patients. We cannot exclude this possibility; however, the fact that also in noradrenaline-prestimulated right atria from β_1-adrenoceptor antagonist-treated patients carbachol exhibited a lower sensitivity although in these atria maximal increase in force of contraction induced by noradrenaline was *not* enhanced argues against this possibility.

It should be noted, however, that such a desensitization of right atrial muscarinic M_2 receptors could not be demonstrated on atria where force of contraction had been increased by 5-HT_4 receptor stimulation, although chronic β_1-adrenoceptor antagonist treatment had sensitized serotonin-induced increase in force of contraction (cf. Fig. 4A). We do not know the reason for this discrepancy; however, the positive inotropic effects of serotonin were—even in atria from patients chronically treated with β_1-adrenoceptor antagonists—rather small. Thus, it might be that in these preparations we might have missed differences because of the rather small *indirect* negative inotropic effects of carbachol.

In conclusion: in isolated electrically driven human right atria, carbachol-induced *indirect* negative inotropic effect is not considerably dependent from the agonist employed to increase (via cyclic AMP accumulation) force of contraction. However, in atria from β_1-adrenoceptor antagonist-treated patients, carbachol-induced *indirect* negative inotropic effect appears to be attenuated.

Acknowledgements

This work was supported by a grant of the Deutsche Forschungsgemeinschaft Bonn/Germany (SFB 598-02 to O.-E.B.). We are thankful to Ciba-Geigy for generous gift of CGP 20712 A.

References

Baumann, G., Mercader, D., Busch, U., Felix, S.B., Loher, U., Ludwig, L., Sebening, H., Heidecke, C.D., Hagl, S., Sebening, F., Blömer, H., 1983. Effects of the H_2-receptor agonist impromidine in human myocardium from patients with heart failure due to mitral and aortic valve disease. J. Cardiovasc. Pharmacol. 5, 618–625.

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.

Borst, M.M., Marquetant, R., Kübler, W., Strasse, R.H., 1997. Beta-blockade reduces effects of adenosine and carbachol by transregulation of inhibitory receptors and G_i proteins. Am. J. Physiol. 272, H1672–H1679.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Brodde, O.-E., 1993. Beta-adrenoceptors in cardiac disease. Pharmacol. Ther. 60, 405–430.

Brodde, O.-E., Michel, M.C., 1999. Adrenergic and muscarinic receptors in the human heart. Pharmacol. Rev. 51, 651–689.

Brodde, O.-E., O'Hara, N., Zerkowski, H.-R., Rohm, N., 1984. Human cardiac β-adrenoceptors: both β_1- and β_2-adrenoceptors are functionally coupled to the adenylate cyclase in right atrium. J. Cardiovasc. Pharmacol. 6, 1184–1191.

Brodde, O.-E., Khamssi, M., Michel-Reher, M., Michel, M.C., 1991. Chronic β_1-blocker treatment differentially regulates β-adrenoceptors and muscarinic receptors, but does not affect the amount of Gi-protein in the human right atrium. Br. J. Pharmacol. 102 (Suppl.), 102P (abstr.).

Brodde, O.-E., Michel, M.C., Zerkowski, H.-R., 1995a. Signal transduction mechanisms controlling cardiac contractility and their alterations in chronic heart failure. Cardiovasc. Res. 30, 570–584.

Brodde, O.-E., Zerkowski, H.-R., Schranz, D., Broede-Sitz, A., Michel-Reher, M., Schäfer-Beisenbusch, E., Piotrowski, J.A., Oelert, H., 1995b. Age-dependent changes of the β-adrenoceptor-G-protein(s)-adenylyl cyclase system in human right atrium. J. Cardiovasc. Pharmacol. 26, 20–26.

Brown, L., Deighton, N.M., Bals, S., Söhlmann, W., Zerkowski, H.-R., Michel, M.C., Brodde, O.-E., 1992. Spare receptors for β-adrenocep-

tor-mediated positive inotropic effects of catecholamines in the human heart. J. Cardiovasc. Pharmacol. 19, 222–232.

Caulfield, M.P., Birdsall, N.J.M., 1998. International Union of Pharmacology XVII. Classification of muscarinic acetylcholine receptors. Pharmacol. Rev. 50, 279–290.

Deighton, N.M., Motomura, S., Borquez, D., Zerkowski, H.-R., Doetsch, N., Brodde, O.-E., 1990. Muscarinic cholinoceptors in the human heart: demonstration, subclassification, and distribution. Naunyn-Schmiedeberg's Arch. Pharmacol. 341, 14–21.

Deighton, N.M., Motomura, S., Bals, S., Zerkowski, H.-R., Brodde, O.-E., 1992. Characterization of the beta adrenoceptor subtype(s) mediating the positive inotropic effects of epinine, dopamine, dobutamine, denopamine and xamoterol in isolated human right atrium. J. Pharmacol. Exp. Ther. 262, 532–538.

Dhein, S., Van Koppen, C.J., Brodde, O.-E., 2001. Muscarinic receptors in the mammalian heart. Pharmacol. Res. 44, 161–182.

Dooley, D.J., Bittiger, H., Reymann, N.C., 1986. CGP 20712 A: a useful tool for quantitating β_1- and β_2-adrenoceptors. Eur. J. Pharmacol. 130, 137–139.

Ferro, A., Plumpton, C., Brown, M.J., 1993. Is receptor cross-regulation in human heart caused by alterations in cardiac guanine nucleotide-binding proteins? Clin. Sci. 85, 393–399.

Giessler, C., Wangemann, T., Zerkowski, H.-R., Brodde, O.-E., 1998. Age-dependent decrease in the negative inotropic effect of carbachol on isolated human right atrium. Eur. J. Pharmacol. 357, 199–202.

Hall, J.A., Kaumann, A.J., Brown, M.J., 1990. Selective β_1-adrenoceptor blockade enhances positive inotropic responses to endogenous catecholamines mediated through β_2-adrenoceptors in human atrial myocardium. Circ. Res. 66, 1610–1623.

Jia, H., Monteith, S., Brown, M.J., 1995. Expression of the α- and β-subunits of the stimulatory and inhibitory G-proteins in β_1-adrenoceptor-blocked and non-β-adrenoceptor-blocked human right atrium. Clin. Sci. 88, 571–580.

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., Hall, J.A., Murray, K.J., Wells, F.C., Brown, M.J., 1989. A comparison of the effects of adrenaline and noradrenaline on human heart: the role of β_1- and β_2-adrenoceptors in the stimulation of adenylate cyclase and contractile force. Eur. Heart. J. 10 (Suppl. B), 29–37.

Kaumann, A.J., Sanders, L., Brown, A.M., Murray, K.J., Brown, M.J., 1991. A 5-HT_4-like receptor in human right atrium. Naunyn-Schmiedeberg's Arch. Pharmacol. 344, 150–159.

Marquetant, R., Brehm, B., Strasser, R.H., 1992. Chronic beta-blockade transregulates inhibitory A_1 adenosine and muscarinic M_2 receptors of the adenylyl cyclase system. J. Mol. Cell. Cardiol. 24, 535–548.

Michel, M.C., Pingsmann, A., Beckeringh, J.J., Zerkowski, H.-R., Doetsch, N., Brodde, O.-E., 1988. Selective regulation of β_1- and β_2-adrenoceptors in the human heart by chronic β-adrenoceptor antagonist treatment. Br. J. Pharmacol. 94, 685–692.

Motomura, S., Deighton, N.M., Zerkowksi, H.-R., Doetsch, N., Michel, M.C., Brodde, O.-E., 1990. Chronic β_1-adrenoceptor antagonist treatment sensitizes β_2-adrenoceptors, but desensitizes M_2-muscarinic receptors in the human right atrium. Br. J. Pharmacol. 101, 363–369.

Salomon, Y., Londos, C., Rodbell, M., 1974. A highly sensitive adenylate cyclase assay. Anal. Biochem. 58, 541–548.

Sanders, L., Lynham, J.A., Bond, B., del Monte, F., Harding, S.E., Kaumann, A.J., 1995. Sensitization of human atrial 5-HT_4-receptors by chronic β-blocker treatment. Circulation 92, 2526–2539.

Sanders, L., Lynham, J.A., Kaumann, A.J., 1996. Chronic β_1-adrenoceptor blockade sensitises the H_1 and H_2 receptor systems in human atrium: role of cyclic nucleotides. Naunyn-Schmiedeberg's Arch. Pharmacol. 353, 661–670.

Schäfers, R.F., Adler, S., Daul, A., Zeitler, G., Vogelsang, M., Zerkowski, H.-R., Brodde, O.-E., 1994. Positive inotropic effect of the $beta_2$-adrenoceptor agonist terbutaline in the human heart: effects of long-term $beta_1$-adrenoceptor antagonist treatment. J. Am. Coll. Cardiol. 23, 1224–1233.

Yamada, M., Inanobe, A., Kurachi, Y., 1998. G protein regulation of potassium ion channels. Pharmacol. Rev. 50, 723–760.

Zerkowski, H.-R., Broede, A., Kunde, K., Hillemann, S., Schäfer, E., Vogelsang, M., Michel, M.C., Brodde, O.-E., 1993. Comparison of the positive inotropic effects of serotonin, histamine, angiotensin II, endothelin and isoprenaline in the isolated human right atrium. Naunyn-Schmiedeberg's Arch. Pharmacol. 347, 347–352.