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Short communication

Age-dependent decrease in the negative inotropic effect of carbachol on isolated human right atrium

Christine Giessler [a], Thekla Wangemann [b], Hans-Reinhard Zerkowski [b], Otto-Erich Brodde [a,*]

[a] *Institute of Pharmacology and Toxicology, Martin-Luther University of Halle-Wittenberg, Magdeburger Str. 4, 06097 Halle / Saale, Germany*
[b] *Clinic for Cardio-Thoracic Surgery, Martin-Luther University of Halle-Wittenberg, Ernst Grube Str. 40, 06097 Halle / Saale, Germany*

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Abstract

On isolated, electrically driven human right atrial strips, carbachol (10^{-8}–10^{-3} M) concentration-dependently decreased force of contraction prestimulated with 1 μM forskolin; maximal negative inotropic effects of carbachol (10^{-6}–3×10^{-6} M), however, were in atria from patients aged < 25 years (mean age: 16.8 ± 2.0 years, $n = 9$) significantly larger than in patients aged 50–69 years (mean age: 62.5 ± 0.7 years, $n = 33$) and were further decreased in patients aged > 70 years (mean age: 73.8 ± 0.6 years, $n = 11$). We conclude that, in human right atrium, the recently described age-dependent decrease in muscarinic M_2 receptor density is accompanied by a decrease in negative inotropic effects.

Keywords: Heart, human; Muscarinic M_2 receptor; Muscarinic M_1 receptor; Ageing, cardiac; Negative inotropic effect; Carbachol

1. Introduction

In ageing the responsiveness of many hormone receptors is altered (Roth, 1997). This holds true also for human cardiac β-adrenoceptors (White et al., 1994; Brodde et al., 1995) and muscarinic receptors (Poller et al., 1997; Brodde et al., 1998). Muscarinic receptors in the human heart are predominantly of the M_2-subtype; they couple to adenylyl cyclase in an inhibitory fashion and mediate negative inotropic effects (Giraldo et al., 1988; Böhm et al., 1990; Deighton et al., 1990). We have recently demonstrated that, in healthy volunteers, decreases in heart rate induced by i.v. application of low doses of atropine or the muscarinic M_1 receptor selective antagonist pirenzepine (Pitschner and Wellstein, 1988; Wellstein and Pitschner, 1988) are attenuated with increasing age (Poller et al., 1997); subsequently we could show that, in human right atrium, muscarinic M_2 receptor density declines with ageing and there was a significant negative correlation between age and muscarinic M_2 receptor density (Brodde et al., 1998). The age-dependent decrease in muscarinic M_2 receptor density was accompanied by an age-dependent decline in the ability of carbachol to inhibit forskolin-stimulated adenylyl cyclase. The aim of the present study was to find out, whether such an age-dependent decrease can be also demonstrated for the negative inotropic effect of carbachol on isolated electrically driven human right atrial trabecular strips.

2. Materials and methods

2.1. Patients

Right atrial appendages were obtained (a) from 9 children (4 female, 5 male) with acyanotic congenital heart disease who underwent open heart surgery because of ventricular septal defect ($n = 2$), atrioventricular septal defect ($n = 2$), and atrial septal defect ($n = 5$) (their parents had given informed written consent). None of the children suffered from acute heart failure or had been treated with sympathomimetics (i.e., catecholamines) or cholinergic drugs for at least 3 weeks before surgery; (b) from 44 adult patients (34 male, 10 female) undergoing elective coronary artery bypass grafting after having given informed written consent; the majority of these patients was without apparent heart failure (NYHA class I–II, $n = 27$) but a few patients were in NYHA class II-III ($n = 9$) or class III ($n = 8$). None of these patients had

* Corresponding author. Tel.: +49-345-557-1773; Fax: +49-345-557-1835.

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been treated with β-adrenoceptor agonists or cholinergic drugs for at least 6 weeks before operation. However, patients had received nitrates ($n = 28$), molsidomine ($n = 26$), β-adrenoceptor antagonists ($n = 22$), Ca^{2+} channel antagonists ($n = 11$), angiotensin-converting enzyme-inhibitors ($n = 25$), diuretics ($n = 11$), digitalis glycosides ($n = 4$), heparine ($n = 2$), acetyl salicylic acid ($n = 12$), allopurinol ($n = 5$), lipid-lowering drugs ($n = 9$), and antibiotics ($n = 4$), alone or in combination.

Anesthesiological premedication and the surgical procedure have been described in details recently (Brodde et al., 1998). Right atrial appendages were removed immediately after installation of the cardiopulmonary bypass.

Patients were arbitrarily divided into three age-groups: young (group A: < 25 years), elder adults (group B: 50–69 years) and old subjects (group C: > 70 years); the mean ages of the three groups were: A: 16.8 ± 2.0 years (range: 6–24 years), $n = 9$; B: 62.5 ± 0.7 years (range: 51–69 years), $n = 33$; C: 73.8 ± 0.6 years (range: 71–78 years), $n = 11$.

2.2. Preparations

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 atria were dissected to yield trabecular strips (4–5 mm long and 1 mm or less in diameter) without endocardial damage. Usually 2–3, sometimes 4 trabecular strips were obtained from each right atrium.

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 (95% O_2/5% CO_2)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 KG, March-Hugstetten, Germany). Propranolol (10^{-7} M) was present throughout the experiments. The developed tension of the preparations (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, contractile force of the strips was enhanced by 1 μM forskolin. When contractile force had reached a steady state level, cumulative concentration–response curves for carbachol (10^{-8}–10^{-3} M) were determined; in order to correct for spontaneous decline in forskolin-induced increase in contractile force, one strip was always run without carbachol. Spontaneous decline in contractile force over the period of a carbachol–concentration–response curve was $11 \pm 0.2\%$ ($n = 53$). For antagonist experiments preparations were equilibrated for 30 min with the antagonists.

2.3. Statistics

The experimental data given in text and figures are expressed as the means ± S.E.M. of n experiments. The significance of differences was estimated by non-paired two-tailed Student's *t*-test or, if appropriate, by repeated measures analysis of variance followed by the *t*-test using Bonferroni corrections for multiple comparisons. A P value smaller than 0.05 was considered to be significant. All statistical calculations were performed with the Instat program (GraphPAD Software, San Diego, USA).

The pD_2-values for carbachol (i.e., the negative logarithm of the molar concentration of carbachol causing half maximal effects) were determined as described by Van Rossum (1963). The equilibrium dissociation constant (K_B) for the muscarinic receptor antagonists was calculated using the formula (Furchgott, 1972):

$$K_B = [A]/(CR - 1)$$

with $[A]$ = concentration of antagonist used, and CR = concentration ratio (i.e., the ratio of equieffective concentrations of carbachol in the presence and absence of antagonist).

2.4. Drugs

Carbachol (carbamylcholine chloride) and (−) propranolol hydrochloride were purchased from Sigma (Deisenhofen, Germany), and forskolin dihydrochloride from Calbiochem-Novabiochem (Bad Soden, Germany). AF-DX 116 (11-[[2-[(diethylamino)methyl]-1-piperidinyl]-acetyl]-5,11,-dihydro − 6*H*-pyrido[2,3-b][1,4] benzodiazepin − 6-one) and pirenzepine dihydrochloride were kindly provided by Dr. Karl Thomae (Biberach a.d. Riss, Germany). All other chemicals were of the highest purity grade commercially available.

3. Results

On the isolated electrically driven human right atrium carbachol caused concentration-dependent decreases in force of contraction previously enhanced by 1 μM forskolin (Fig. 1); maximal effects were reached at 3×10^{-6} M carbachol; the pD_2-value was 6.76 ± 0.06 ($n = 33$). The muscarinic M_2 receptor selective antagonist AF-DX 116 (10^{-6} M) caused a significant rightward shift of the carbachol concentration-response curve (Fig. 1); from this shift a pK_B-value of 7.11 ± 0.05 ($n = 17$) was calculated. In the same concentration (10^{-6} M) the muscarinic M_1 receptor selective antagonist pirenzepine caused a significant smaller rightward-shift of the concentration-response curve for carbachol (Fig. 1); the resulting pK_B-value for pirenzepine was 6.63 ± 0.13 ($n = 18$).

In concentrations $> 3 \times 10^{-6}$ M, carbachol increased contractile force; this effect was nearly completely sup-

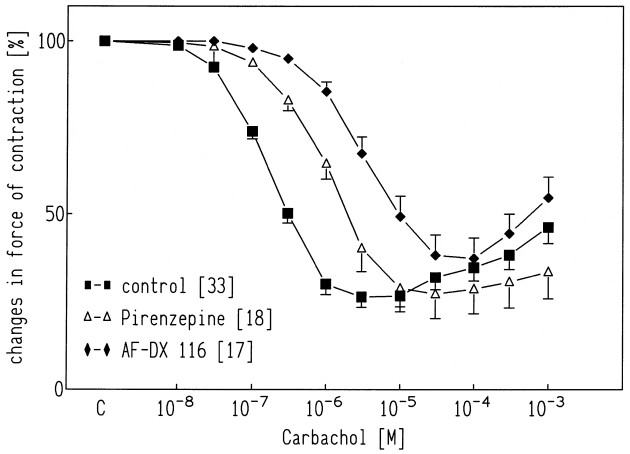

Fig. 1. Inhibition by pirenzepine (10^{-6} M, △–△) and AF-DX 116 (10^{-6} M, ◆–◆) of the negative inotropic effect of carbachol (■–■) on isolated electrically driven human right atrial trabecular strips prestimulated with 10^{-6} M forskolin. Ordinate: Negative inotropic effect in % of maximal force of contraction (i.e., force of contraction in the presence of 10^{-6} M forskolin). Abscissa: molar concentrations of carbachol. Basal force of contraction amounted to 2.9 ± 0.4 mN, in the presence of 10^{-6} M pirenzepine to 3.7 ± 0.6 mN and in the presence of 10^{-6} M AF-DX 116 to 3.0 ± 0.4 mN; maximal force of contraction in the presence of 10^{-6} M forskolin was 4.6 ± 0.4 mN, in the presence of 10^{-6} M pirenzepine 5.3 ± 0.6 mN and in the presence of 10^{-6} M AF-DX 116 4.5 ± 0.5 mN. Means ± S.E.M.; number of experiments (= number of patients studied) in brackets.

pressed by pirenzepine (10^{-6} M), but not affected by AF-DX 116 (10^{-6} M, Fig. 1).

However, the maximal negative inotropic effect induced by carbachol was in group A (aged < 25 years) significantly larger than in group B (aged 50–69 years) and was further attenuated in group C (aged > 70 years, Fig. 2); pD_2-Values for carbachol were, however, not changed and amounted in group A to 6.86 ± 0.14, in group B to 6.77 ± 0.06 and in group C to 6.76 ± 0.15. The increase in force of contraction, induced by 10^{-6} M forskolin, was similar in right atria from group A (1.64 ± 0.11 mN, $n = 9$) and group B (1.6 ± 0.13, $n = 33$) but significantly less ($p < 0.05$) in group C (1.08 ± 0.14 mN, $n = 11$).

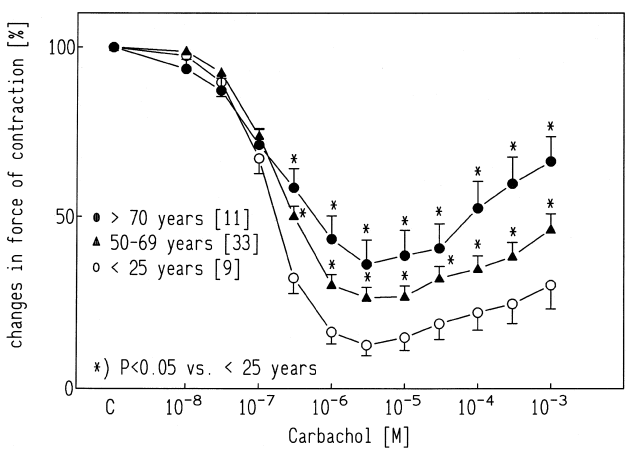

Fig. 2. The negative inotropic effect of carbachol on isolated electrically driven human right atrial trabecular strips obtained from 9 patients aged < 25 years (group A), 33 patients aged 50–69 years (group B) and 11 patients aged > 70 years (group C) prestimulated with 10^{-6} M forskolin. Ordinate: Negative inotropic effect in % of maximal force of contraction (i.e., force of contraction in the presence of 10^{-6} M forskolin). Abscissa: molar concentrations of carbachol. Basal force of contraction amounted in group A to 2.3 ± 0.4 mN, in group B to 2.9 ± 0.4 mN and in group C to 2.1 ± 0.5 mN; maximal force of contraction in the presence of 10^{-6} M forskolin was in group A 4.0 ± 0.4 mN, in group B 4.6 ± 0.4 and in group C 3.2 ± 0.5 mN. Means ± S.E.M.; number of experiments (= number of patients studied) in brackets.

In a few atria we were able to test the effects of carbachol on basal force of contraction. In agreement with our previously reported data (Deighton et al., 1990) carbachol (10^{-8}–10^{-3} M) caused concentration-dependent decreases in basal force of contraction; maximal decrease in basal force of contraction at 10^{-6} M carbachol, however, showed the same age-dependent pattern as described above: thus, in 2 patients aged < 25 years it was $88.1 \pm 6\%$, in 4 patients aged 50–69 years it was $73.5 \pm 11\%$ and in 3 patients aged > 70 years it was $48.6 \pm 17\%$.

4. Discussion

We have recently shown that, in human right atria, muscarinic receptor number declines with ageing; this was accompanied in vitro by a reduced ability of carbachol to inhibit forskolin-stimulated adenylyl cyclase activity and in vivo, in healthy volunteers, by a reduced decrease in resting heart rate as well as in isoprenaline-stimulated heart rate evoked by low dose i.v. application of the muscarinic M_1 receptor antagonist pirenzepine (Poller et al., 1997; Brodde et al., 1998). The present results confirm and extend these observations: they clearly show that, in human right atrium, the negative inotropic effect of carbachol decreases with increasing age—and this appears to be true for force of contraction prestimulated with forskolin as well as for basal force of contraction. In accordance with previously reported data (Giraldo et al., 1988; Deighton et al., 1990), the negative inotropic effect of carbachol appears to be mediated by muscarinic M_2 receptor activation, since it was antagonized by the muscarinic M_2 receptor antagonist AF-DX 116 with a pK_B-value (7.11 ± 0.05) which was well in the affinity range of AF-DX 116 for muscarinic M_2 receptors (Eglen et al., 1996). In addition, the muscarinic M_1 receptor selective antagonist pirenzepine was only a weak antagonist with a rather low pK_B-value (6.63 ± 0.13); this pK_B-value was well in its affinity range for muscarinic M_2 receptors but 1–2 orders of magnitude less than its affinity for muscarinic M_1 receptors (Eglen et al., 1996).

In our study some patients with moderate heart failure were investigated. Thus, it could be that part of the age-dependent decline in carbachol effects might be disease-induced rather than ageing-induced. We cannot completely rule out that possibility; however, we believe that this is quite unlikely because several groups have shown,

that in the failing human heart muscarinic receptor density and the negative inotropic effect of carbachol is unchanged when compared with non-failing hearts (Böhm et al., 1990; Brodde et al., 1992; Bristow, 1993)

It should be noted that—at least for groups A and B—the forskolin-induced increase in force of contraction was nearly identical; thus, differences in carbachol-induced negative inotropic effects are not due to different levels of prestimulated force of contraction. In the group of patients elder than to 70 years, however, forskolin-stimulated increase in force of contraction was diminished, presumably due to the fact that with increasing age the activity of the catalytic unit of adenylyl cyclase decreases (Brodde et al., 1995) and forskolin evokes its action predominantly via direct activation of the adenylyl cyclase (Seamon and Daly, 1986). This may contribute to the markedly reduced negative inotropic effect of carbachol observed in the old subjects in this study.

In conclusion, in human right atrium, the recently described age-dependent decrease in muscarinic M_2 receptor density (Brodde et al., 1998) is accompanied by an age-dependent decrease in the negative inotropic effect evoked by carbachol - and this holds true for basal force of contraction as well as for force of contraction prestimulated with 1 μM forskolin.

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