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Review

Muscarinic receptors in the failing human heart

Christine Giessler, Stefan Dhein, Klaus Pönicke, Otto-Erich Brodde *

Institute of Pharmacology and Toxicology, Martin-Luther-University of Halle, Wittenberg, Magdeburger Str. 4, D-06097 Halle, Germany

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Abstract

In the human heart, as in the heart of several other species, muscarinic receptors are predominantly of the M_2-subtype that couple via a pertussis toxin-sensitive G_i-protein to inhibit adenylyl cyclase. However, it is not clear whether an additional muscarinic receptor subtype exists in the human heart. In human right atrium, stimulation of muscarinic M_2 receptors causes direct negative inotropic and chronotropic effects; in human ventricular myocardium, however, the negative inotropic effect can be only achieved when basal force of contraction has been pre-stimulated by cyclic AMP-elevating agents such as β-adrenoceptor agonists, forskolin or phosphodiesterase inhibitors (indirect effect); this has been shown in various in vitro and in vivo studies. Evidence has accumulated that in chronic heart failure vagal activity is decreased. Cardiac muscarinic M_2 receptor density and functional responsiveness (inhibition of adenylyl cyclase activity and negative inotropic effects), however, are not considerably changed when compared with non-failing hearts although cardiac G_i-activity is increased.

Keywords: Muscarinic M_2 receptor; Heart human; Chronic heart failure; Adenylyl cyclase; Negative inotropic effect

One of the characteristics and well-known alterations in patients with chronic heart failure is an increase in the activity of the sympathetic nervous system. In patients with heart failure, plasma noradrenaline levels are elevated (Cohn, 1995). Moreover, cardiac noradrenaline spillover is increased due to enhanced cardiac sympathetic drive (for recent review see Esler et al., 1997) and decreased cardiac neuronal uptake (Böhm et al., 1995; Eisenhofer et al., 1996); in addition, cardiac noradrenaline stores are depleted (Chidsey and Braunwald, 1966; Anderson et al., 1992).

The increased cardiac drive that appears to occur very early in heart failure (Rundqvist et al., 1997) might be responsible for two well characterized abnormalities in the failing human heart: a desensitization of the cardiac β-adrenoceptor system (that in the human heart, is the most powerful physiologic system to acutely increase force of contraction and heart rate, Brodde et al., 1995) and toxic effects on cardiomyocytes (Mann et al., 1992). Alterations of the β-adrenoceptor-G-protein(s)-adenylyl cyclase system in chronic heart failure have been studied very intensively during the last 15 years. It is now generally accepted that, in patients with chronic heart failure, there is a substantial decrease in cardiac β_1-adrenoceptors (that occurs on a protein- and mRNA-level), an uncoupling of cardiac β_2-adrenoceptors (but often no change in number or mRNA-levels), no change in the amount and functional activity of cardiac G_s, an upregulation of the activity (and in most but not all studies) amount of cardiac G_i, an upregulation of mRNA levels and phosphorylation activity of cardiac β-adrenoceptor kinase and no change in the activity of the catalytic unit of adenylyl cyclase and of the cyclic AMP-dependent protein kinase A (for reviews see Feldman and Bristow, 1990; Brodde, 1991; Bristow, 1993; Brodde, 1993; Harding et al., 1994; Böhm, 1995; Brodde et al., 1995; Bristow, 1997; Ferrara et al., 1997). Since the human heart has only a few, if any, 'spare' β-adrenoceptors (Brown et al., 1992) it is plausible that these changes are accompanied by a decreased cardiac β-adrenoceptor functional responsiveness that has been demonstrated in numerous in vitro (on isolated cardiac preparations) and in vivo studies. Moreover, a recent study in atrial and ventricular preparations of end-stage failing human hearts demonstrated that not only responses to β-adrenoceptor stimulation but also to stimulation of other G_s-coupled receptors such as histamine or serotonin were diminished (Brodde et al., 1998b) which is presumably due to the increased

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

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activity of G_i that mitigates cyclic AMP formation; this may also explain why the effects of phosphodiesterase inhibitors on force of contraction are diminished in the failing human heart (Feldman et al., 1987).

On the other hand, only little is known on possible changes in cardiac muscarinic receptors in chronic heart failure. In the human heart, as in the heart of several other species (for review see Caulfield, 1993) muscarinic receptors are predominantly of the M_2-subtype (Giraldo et al., 1988; Deighton et al., 1990) that couple via a pertussis toxin-sensitive G_i-protein to inhibition of adenylyl cyclase (Felder, 1995; Wess, 1996). In human right atrium, stimulation of muscarinic M_2 receptors causes direct negative inotropic and chronotropic effects; in ventricles, however, the negative inotropic effect can be only achieved when basal force of contraction has been pre-stimulated by cyclic AMP-elevating agents such as β-adrenoceptor agonists, forskolin or phosphodiesterase inhibitors (indirect effect or 'accentuated antagonism', Levy, 1971); this has been shown in various in vitro (Delhaye et al., 1984; Jakob et al., 1989; Böhm et al., 1990a; Deighton et al., 1990; Motomura et al., 1990; Ungerer et al., 1990; Böhm et al., 1994; Du et al., 1994; Koglin et al., 1994; Du et al., 1995; Giessler et al., 1998) and in vivo studies (Von Scheidt et al., 1992; Koglin et al., 1994; Landzberg et al., 1994; Newton et al., 1996).

In both atrial and ventricular myocardium activation of muscarinic M_2 receptors leads via G_i to inhibition of adenylyl cyclase and, by this, to a decrease in the formation of intracellular cyclic AMP which in turn results in a reduction in the L-type Ca^{2+} current (Giles and Noble, 1976) and appears to be the predominant mechanism of the negative inotropic effect of acetylcholine (indirect effect or 'accentuated antagonism'). In addition, however, muscarinic M_2 receptor-mediated activation of cyclic GMP-dependent protein kinase G or activation of cyclic GMP-stimulated cyclic AMP phosphodiesterase (for recent review see Mery et al., 1997) and/or stimulation of phosphatase activity (Herzig et al., 1995) might contribute to the indirect negative inotropic effect of acetylcholine. In atria, however, the direct negative inotropic effect is due to an activation of inwardly rectifying potassium channel (I_{KACh}) via direct effects of the G-protein α-(for recent review see Clapham and Neer, 1997) or (preferentially?) βγ-subunits (for recent review see Yamada et al., 1998). On the contrary, in ventricular myocardium acetylcholine has no direct negative inotropic effect and it is still a matter of debate whether or not acetylcholine might activate the I_{KACh} in ventricular myocardium (Koumi et al., 1997).

In addition to indirect and direct negative inotropic effects muscarinic receptor stimulation by high concentrations of acetylcholine or carbachol (usually $> 10^{-6}$–10^{-5} M) causes a positive inotropic effect. This effect is pertussis toxin-insensitive, often only seen after pertussis toxin-treatment and it has been speculated that it is linked to the phospholipase C/inositol-trisphosphate/diacyl-glycerol pathway (for review see Caulfield, 1993; Mery et al., 1997). It is, however, still a matter of debate whether inositol phosphate formation and positive inotropic effects induced by acetylcholine or carbachol are mediated by muscarinic M_2- or another muscarinic non-M_2-receptor, possibly muscarinic M_1 receptor (Sharma et al., 1997).

Evidence has accumulated that also in the human heart there might exist an additional muscarinic receptor that is not of the M_2-subtype. Thus, as in the heart of other mammalian species, in human atrial and ventricular preparations higher concentrations of acetylcholine or carbachol increased force of contraction (Du et al., 1995; Giessler et al., 1998). In the atria this effect was much more sensitive to the muscarinic M_1 receptor antagonist pirenzepine than to the muscarinic M_2 receptor antagonist AF-DX 116 (11-[{2-[(diethylamino)-methyl]-1-piperidyl}-acetyl)-5,11-dihydro-6*H*-pyridol[2,3-b][1,4]benzodiazepine-6-one)(Du et al., 1995; Giessler et al., 1998) while in the ventricular preparations AF-DX 116 was a much more potent antagonist than pirenzepine (Du et al., 1995). Moreover, carbachol has been found to increase inositol phosphate formation in the human heart (Bristow, 1993). And finally, several groups have shown that, in vivo in humans, low ('selective') doses of the muscarinic M_1 receptor antagonist pirenzepine cause negative chronotropic effects while only higher ('non-selective') doses cause increases in heart rate (Meyer and De Sommers, 1988; Pitschner and Wellstein, 1988; Poller et al., 1997); similar effects were also obtained with atropine: in low doses it decreased heart rate while in higher doses it increased heart rate (Wellstein and Pitschner, 1988; Poller et al., 1997). On the other hand, the selective muscarinic M_2 receptor antagonist AF-DX 116 only increased heart rate in healthy volunteers (Schulte et al., 1991). These results strongly indicate that the muscarinic receptor subtype involved in the 'cholinomimetic' effects of pirenzepine and atropine is not a muscarinic M_2 receptor but rather an additional muscarinic receptor, very likely a muscarinic M_1 receptor (Brodde et al., 1998a). Taken together, at least in human right atrium the existence of a second muscarinic receptor subtype in addition to the muscarinic M_2 receptor is quite likely; however, the final experimental proof is still lacking.

Only a few attempts have been made to study possible alterations in cardiac muscarinic receptors in patients with chronic heart failure. Anti-muscarinic M_2 receptor autoantibodies have been described in patients with idiopathic dilated cardiomyopathy (Fu, 1996) and patients with Chagas' disease (Leiros et al., 1997) but their pathophysiological role is still a matter of debate. In patients with end-stage heart failure due to idiopathic dilated or to ischemic cardiomyopathy Böhm et al. (1990a,b) have shown that the density of ventricular muscarinic receptors was not changed while in the same patients β-adrenoceptor numbers were significantly reduced. Similar results were obtained by Fu et al. (1992) who also found unchanged ventricular mus-

carinic M_2 receptor densities but decreased β-adrenoceptor densities in patients with end-stage dilated cardiomyopathy in comparison to non-failing hearts. In a recent in vivo positron emission tomography (PET) study using [^{11}C]methylquinuclidinyl benzilate as ligand, however, Le Guludec et al. (1997) found cardiac muscarinic receptors to be slightly but significantly higher in 20 patients with congestive heart failure than in 12 healthy controls. On the other hand, the negative inotropic effect of carbachol on isolated, electrically driven papillary muscle strips that had been pre-stimulated with forskolin (Böhm et al., 1990a) or isoprenaline (Schmitz et al., 1996) were superimposable in patients with chronic heart failure and non-failing controls; in isolated atrial myocytes from failing human hearts, however, a reduced I_{KACh} sensitivity to muscarinic M_2 receptor linked G_i-protein was found (Koumi et al., 1994).

Studies in experimental animal models of heart failure have resulted in divergent results: in a dog model of left ventricular failure due to aortic banding (pressure-overload) cardiac muscarinic receptor density and functional responsiveness was found to be reduced, while cardiac G_i was unchanged (Vatner et al., 1988). Similar results were obtained in rats with aortic banding and large cardiac hypertrophy: cardiac muscarinic receptor density and functional responsiveness were decreased when compared with sham-operated rats (Mertens et al., 1995). Moreover, in ethanol-induced heart failure in the rat a reduced expression of cardiac muscarinic receptors was found (Strasser et al., 1996). On the other hand rapid pacing induced heart failure in dogs was found to be accompanied by an increase in cardiac G_i and an increase in ventricular, but not atrial muscarinic M_2 receptor density (Wilkinson et al., 1996), an increased inhibition of isoprenaline-stimulated adenylyl cyclase by carbachol and an increased inhibition of contractile force by acetylcholine (Vatner et al., 1996).

In order to get further insight into possible changes of human cardiac muscarinic receptors in chronic heart failure, we have recently studied muscarinic receptor density and function in right atria and left ventricles from patients with end-stage heart failure undergoing heart transplantation; right atrial appendages obtained from patients undergoing coronary artery bypass grafting without apparent heart failure and left ventricular myocardium from non-failing hearts obtained from potential organ donors whose hearts could not be transplanted for technical reasons served as controls (Pönicke et al., 1998).

In these hearts we assessed muscarinic receptor density (by [^{3}H]*N*-methyl-scopolamine binding, as recently described, Brodde et al., 1998a), carbachol-induced inhibition of isoprenaline- and forskolin-stimulated adenylyl cyclase activity (for details see Pönicke et al., 1998), and on isolated, electrically driven left ventricular trabecular strips inhibition of forskolin-stimulated force of contraction by carbachol (as recently described by Giessler et al., 1998). For reason of comparison, β-adrenoceptor densities (by (−)-[^{125}I]iodocyanopindolol binding as recently described, Brodde et al., 1998b) were assessed in right atria and left ventricles.

Fig. 1 shows that in right atrial membranes muscarinic receptor density was nearly identical in failing and non-failing hearts. It is important to note that the mean age of the patients involved in this study (53 ± 2 years, non-failing vs. 62 ± 2.5 years, failing) was comparable to exclude possible age-dependent decreases in muscarinic receptor density, as recently described (Brodde et al., 1998a). In left ventricular membranes muscarinic receptor density in the failing hearts tended to be higher than in non-failing hearts (although the mean age in the non-failing group [33 ± 4 years] was markedly lower than in the group of patients with heart failure [51 ± 2 years]), but this difference did not reach statistical significance. On the other hand, right atrial and left ventricular β-adrenoceptor densities were in the failing hearts significantly lower than in non-failing hearts (Fig. 1). Thus, these data confirm those published in the literature (see above) that, in the severely failing human heart, muscarinic receptor density is *not* decreased, in contrast to β-adrenoceptors which are markedly decreased.

In right atrial and left ventricular membranes of the failing hearts 10 μM GTP- (acting at G_s- and G_i-protein) and 10 μM isoprenaline-activated adenylyl cyclase activity was significantly reduced compared to non-failing hearts, whereas activation of adenylyl cyclase by 10 mM NaF (acting at G_s) and 10 mM Mn^{2+}-ions (acting only at the catalytic unit of the enzyme) was not different between failing and non-failing hearts; activation of adenylyl cyclase by 10 μM forskolin (acting predominantly at the catalytic unit of the enzyme but involving partly G_s) tended to be lower in the failing hearts than in the non-failing hearts but this difference did not reach statistical significance (Fig. 2). These results (reduced adenylyl cy-

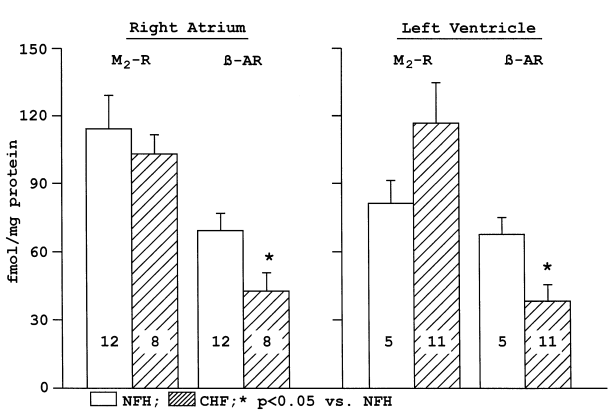

Fig. 1. Muscarinic receptor density and β-adrenoceptor density in right atria and left ventricular membranes of non-failing (NFH) and severely failing human hearts (CHF). Ordinates: Muscarinic receptor (M_2-R) density in femtomoles [^{3}H]*N*-methyl-scopolamine specifically bound/mg protein, and β-adrenoceptor (β-AR) density in femtomoles (−)-[^{125}I]iodocyanopindolol specifically bound/mg protein, respectively. Given are means ± S.E.M. Number of experiments at the bottom of the columns.

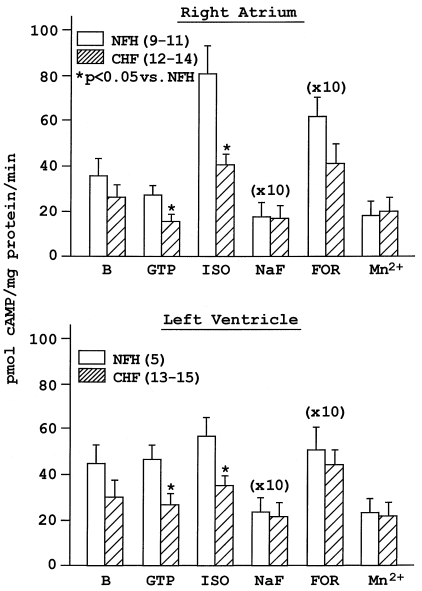

Fig. 2. Adenylyl cyclase activity in membranes from the right atria and left ventricles of non-failing (NFH) and severely failing human hearts (CHF). Ordinates: net increase in right atrial adenylyl cyclase activity upon stimulation in picomoles cAMP formed per milligram protein per minute. Given are means ± S.E.M.; number of experiments in parentheses. B = Basal adenylyl cyclase activity; GTP = 10 μM GTP-B; ISO = 10 μM isoprenaline-GTP; NaF = 10 mM NaF-B; FOR = 10 μM forskolin-GTP; Mn^{2+} = 10 mM Mn^{2+}-B. $^{*}P < 0.05$ vs. NFH. Modified from Pönicke et al. (1998).

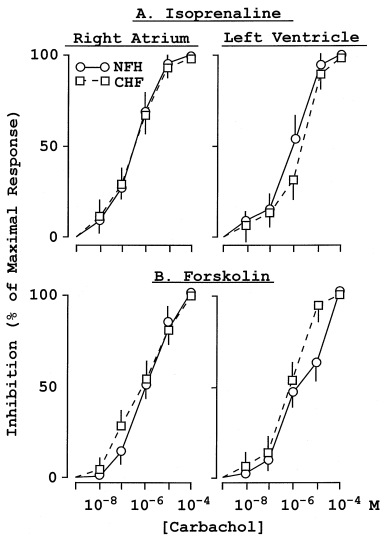

Fig. 3. Inhibition of 10 μM isoprenaline (upper panel) and 10 μM forskolin (lower panel)—stimulated adenylyl cyclase activity by carbachol in membranes from the right atria and left ventricles of non-failing (NFH) and severely failing human hearts (CHF). Ordinate: Inhibition of adenylyl cyclase activity in percent of maximal response (= 100%). Abscissa: molar concentrations of carbachol. Given are means ± S.E.M. Maximal inhibition of isoprenaline-stimulated adenylyl cyclase by carbachol was in NFH-atria 92 ± 17% ($n = 9$), in CHF-atria 80 ± 14% ($n = 13$), in NFH-ventricles 65 ± 15% ($n = 5$), in CHF-ventricles 59 ± 11% ($n = 14$); maximal inhibition of forskolin-stimulated adenylyl cyclase by carbachol was in NFH-atria 32.5 ± 4.5% ($n = 9$), in CHF-atria 22 ± 5% ($n = 13$), in NFH-ventricles 18 ± 2% ($n = 5$), in CHF-ventricles of 19 ± 3.5% ($n = 14$). Data from Pönicke et al. (1998).

clase response to GTP acting at G_s and G_i and unchanged responses to NaF, acting solely at G_s, and to Mn^{2+}, acting at the catalytic unit of adenylyl cyclase), that are in good agreement with the literature (Böhm et al., 1990a; Fu et al., 1992; Bristow, 1993; Eschenhagen, 1993; Brodde et al., 1998b), are in favor of the idea that in the failing hearts the activity of G_i is increased. However, although the activity of G_i was increased inhibition of 10 μM isoprenaline- and 10 μM forskolin-stimulated adenylyl cyclase activity by carbachol was nearly identical in right atrial and left ventricular membranes from failing and non-failing hearts (Fig. 3).

Finally, we determined the negative inotropic effect of carbachol on isolated electrically driven left ventricular trabecular strips from patients with end-stage heart failure and from non-failing hearts. In these experiments force of contraction was pre-stimulated with 1 μM forskolin. Carbachol (10^{-8}–10^{-5} M) caused a concentration-dependent decrease in force of contraction; maximum decrease in force of contraction (Fig. 4) and the pD_2-values for the negative inotropic effect of carbachol were nearly identical in left ventricular trabeculae from failing ($pD_2 = 6.69 \pm 0.09$, $n = 9$) and non-failing hearts ($pD_2 = 6.62 \pm 0.08$, $n = 4$).

Thus, taken these and the results from the literature together it appears that, in the failing human heart, number and function (as assessed by inhibition of adenylyl cyclase activity and negative inotropic effects in isolated cardiac preparations) of muscarinic receptors are *not considerably* changed (Böhm et al., 1990a,b; Fu et al., 1992; Bristow, 1993; Pönicke et al., 1998), although evidence has accumulated that vagal activity is decreased in chronic heart failure (Eckberg et al., 1971; Porter et al., 1990; La Rovere et al., 1994). Similarly, Newton et al. (1996) have recently shown that in patients with chronic heart failure intracoronary infusion of acetylcholine inhibited the left ventricular

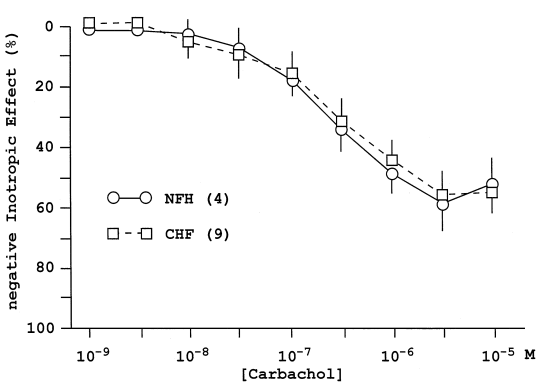

Fig. 4. The negative inotropic effect of carbachol on isolated electrically driven human left ventricular trabecular strips obtained from nine patients with end-stage heart failure (CHF) and from four non-failing hearts (NFH) pre-stimulated with 10^{-6} M forskolin. Ordinate: Negative inotropic effect in percent; Abscissa: molar concentrations of carbachol. Given are means ± S.E.M; Number of experiments in parentheses.

+dP/dT-response to intracoronary dobutamine to nearly the same extent as in patients with normal ventricular function without apparent heart failure. The lack of any changes of human cardiac muscarinic receptors in chronic heart failure is somewhat surprising because human cardiac muscarinic M_2 receptors couple to G_i, and cardiac G_i-activity is increased in chronic heart failure (see above). Thus, the role of increased cardiac G_i-activity in chronic heart failure is still not clear (for discussion see Brodde et al., 1995). In this context it is interesting to note that Eschenhagen et al. (1996) recently showed that, in rats, chronic treatment with carbachol decreased not only cardiac muscarinic M_2 receptor number but also ventricular G_i-content. This was accompanied by a marked increase in isoprenaline- and forskolin-induced arrhythmias in electrically driven papillary muscles. On the other hand, chronic treatment of the rats with isoprenaline, that causes increases in ventricular G_i, rather decreased the incidence in isoprenaline- and forskolin-induced arrhythmias. These results could be taken as a first indication that the increase in the activity of cardiac G_i, often seen in chronic heart failure, might be protective for the heart against catecholamine-induced arrhythmias.

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