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Adrenergic and Muscarinic Receptors in the Human Heart

OTTO-ERICH BRODDE[1] AND MARTIN C. MICHEL

Institute of Pharmacology and Toxicology, University of Halle-Wittenberg, Halle, Germany (O.-E.B.); and Department of Medicine, University of Essen, Germany (M.C.M.)

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I. Introduction

Cardiac function is controlled by the autonomic nervous system (i.e., the sympathetic and the parasympathetic nervous systems), which act via adrenoceptors and muscarinic acetylcholine receptors, respectively. At least nine adrenoceptor subtypes and five muscarinic receptor subtypes exist. In recent years, it has been attempted to associate individual cardiac functions with individual receptor subtypes to further physiological understanding and to identify potential targets for a more specific drug treatment of cardiac disease. The autonomic control of cardiac function can be dynamically regulated by physiological factors such as aging and by disease states such as congestive heart failure. Moreover, interindividual differences may exist due to receptor gene polymorphisms. This article reviews the presence and function of adrenoceptor and muscarinic receptor subtypes in the human heart, as well as their physiological and pathophysiological regulation. Insights into autonomic control of cardiac function from receptor knockout and transgenic animals also are discussed.

[1] Address for correspondence: Otto-Erich Brodde, Ph.D., Institute of Pharmacology and Toxicology, Martin-Luther-University Halle-Wittenberg, Magdeburger Strasse 4, D-06097 Halle/Saale, Germany.

II. Presence and Function of Receptor Subtypes in Human Heart

A. α_1-Adrenoceptors

Three subtypes of α_1-adrenoceptors have been identified pharmacologically and through molecular cloning: α_{1A} (formerly α_{1c}), α_{1B}, and α_{1D} (formerly $\alpha_{1a/d}$; Fig. 1; Hieble et al., 1995; Michel et al., 1995). They are encoded by distinct genes that are located on human chromo-

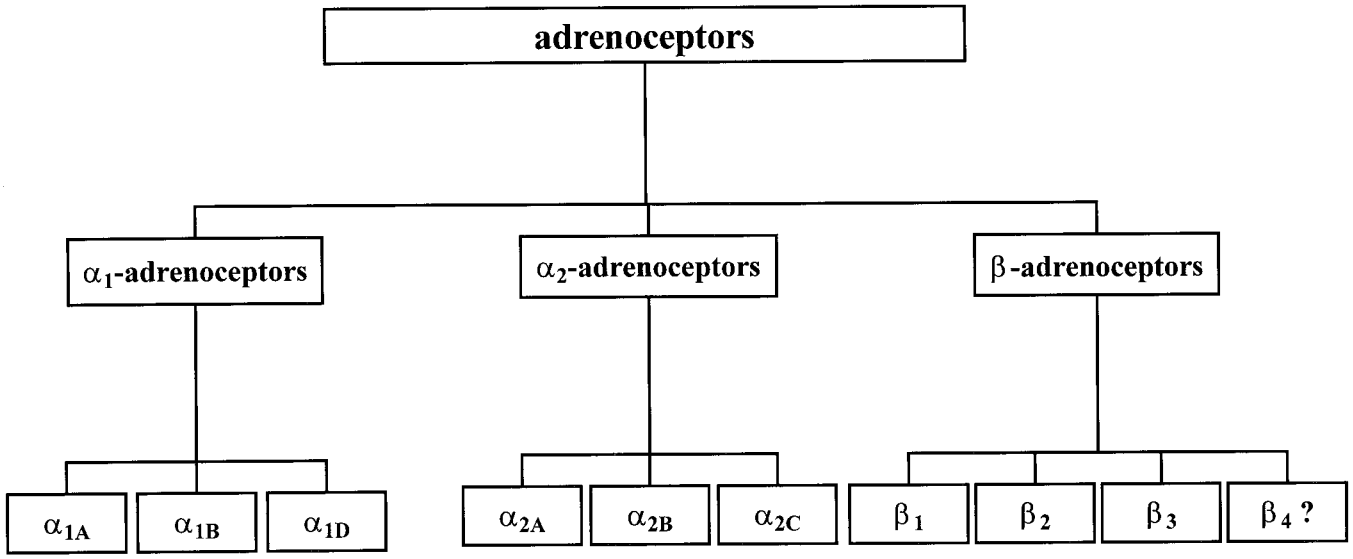

FIG 1. Adrenoceptor subtypes.

TABLE 1
Pharmacological characterization of α_1-adrenoceptor subtypes

	α_{1A}	α_{1B}	α_{1D}
Potency order		noradrenaline = adrenaline[a]	
Selective agonists	A61603		
Selective antagonists	KMD 3213 (10.4) (+)-Niguldipine (10.0) SNAP 5089 (9.7) 5-Methylurapidil (9.2) RS 17053 (9.2) SNAP 5272 (8.4)	AH 11110A (7.1) Chloroethylclonidine[b]	BM Y7378 (8.4)

Antagonist affinities (number in parentheses) are expressed as approximate $-\log K_i$ values.
[a] Both noradrenaline and adrenaline have about 10-fold higher affinity for α_{1D}- than for α_{1A}- and α_{1B}-adrenoceptors.
[b] Although the rank order of α_1-adrenoceptor subtype inactivation by chloroethylclonidine is $\alpha_{1B} \geq \alpha_{1D} > \alpha_{1A}$, chloroethylclonidine can inactivate all α_1-adrenoceptor subtypes depending on concentration and time, temperature, and medium for incubation. Adapted from Alexander and Peters (1999).

somes 8, 5q33, and 20p13, respectively. Further α_1-adrenoceptor heterogeneity is generated by the existence of splice variants of the α_{1A}-adrenoceptor, which differ in their length and sequences of the C-terminal domains (Hirasawa et al., 1995; Chang et al., 1998). All four splice variants can be translated into functional, phospholipase C (PLC)[2]-coupled receptors and have a similar pharmacological recognition profile in competition radioligand binding studies on transfection into Chinese hamster ovary cells (Hirasawa et al., 1995). Because the C-terminal domain of the α_1-adrenoceptors contains amino acids that are believed to be important for receptor desensitization processes (Lattion et al., 1994), it can be speculated that the splice variants of the α_{1A}-adrenoceptor may exhibit differential susceptibility to downregulation, but this has not been tested experimentally. Some studies have proposed the existence of a fourth α_1-adrenoceptor subtype that is primarily characterized by a relatively low affinity for prazosin and some other compounds and therefore was designated α_{1L} (Muramatsu et al., 1990). However, despite extensive efforts, this putative subtype has not been cloned. Recent evidence suggests that it may be a functional state of the α_{1A}-adrenoceptor (Ford et al., 1997). The pharmacological characteristics of α_1-adrenoceptor subtypes are shown in Table 1.

In the human heart, the presence of α_1-adrenoceptors was examined at the mRNA level through RNase protection assays and reverse transcription-polymerase chain reaction (RT-PCR). mRNA for the α_{1B}-adrenoceptor has been detected with RT-PCR (Ramarao et al., 1992; Faure et al., 1995), and small amounts were seen in RNase protection assays in one (Price et al., 1994b) but not another (Weinberg et al., 1994) study. Similarly, mRNA for the α_{1D}-adrenoceptor was found with RT-PCR (Faure et al., 1995) and in RNase protection assays in one (Price et al., 1994b) but not another (Weinberg et al., 1994) study. However, all available studies agree that the α_{1A}-adrenoceptor is the most abundant α_1-adrenoceptor subtype in the human heart at the mRNA level

[2] Abbreviations: PLC, phospholipase C; GRK, G protein-coupled receptor kinase; $I_{K.ACh}$, acetylcholine-activated K^+ channel; NO, nitric oxide; NOS, nitric oxide synthase; PKA, cAMP-dependent protein kinase; L-NMMA, N^G-monomethyl-L-arginine; PKC, protein kinase C; PTX, pertussis toxin; RT, reverse transcription; PCR, polymerase chain reaction; WT, wild-type; SNAP, *S*-nitroso-*N*-acetyl-DL-penicillamine.

(Hirasawa et al., 1993; Price et al., 1994b; Weinberg et al., 1994; Faure et al., 1995). Moreover, all three splice variants of the α_{1A}-adrenoceptor exist in the human heart, although with differing abundance (Hirasawa et al., 1995; Chang et al., 1998). The functional relevance of the α_{1A}-adrenoceptor splice variants in the human heart, as in other tissues, remains to be tested. Although little is known about a possible differential distribution of α_1-adrenoceptor subtypes in various parts of the human heart, studies in rats indicate that the relative abundance of the three subtypes at the mRNA level is similar in all parts of the heart (Wolff et al., 1998). However, it should be noted that an extrapolation from the rat to the human heart may not be possible because α_{1B}- rather than α_{1A}-adrenoceptors have the greatest relative abundance in rat heart (Price et al., 1994a; Wolff et al., 1998) and because the overall expression of functional cardiac α_1-adrenoceptors in rats is much greater than that in humans (see below).

Although the presence of mRNA can predict the presence of corresponding protein in many cases, this has not always been the case with regard to α_1-adrenoceptor subtypes. Thus, the detection of α_1-adrenoceptor protein in the human heart by radioligand binding studies has not been easy despite the presence of large amounts of corresponding mRNA. Nevertheless, several groups of investigators have detected small numbers of human cardiac α_1-adrenoceptors in the right and left ventricles of the human heart (Böhm et al., 1988; Bristow et al., 1988; Limas et al., 1989a; Vago et al., 1989; Steinfath et al., 1992a,b; Hwang et al., 1996). Whenever α_1- and β-adrenoceptor densities were compared within the same study, the latter was always by far more abundant (Böhm et al., 1988; Bristow et al., 1988; Steinfath et al., 1992b; Fig. 2). In a direct comparative study, the human right and left ventricles had the smallest α_1-adrenoceptor density among seven species, including rat, guinea pig, mouse, rabbit, pig, and calf; in contrast, rats had the highest cardiac α_1-adrenoceptor density, which exceeded that of all other species by at least 5-fold (Steinfath et al., 1992a; Fig. 3). These data indicate that the high α_1-adrenoceptor density in rat ventricles may be a particular feature of that species and necessitate great care in extrapolation of rat data to the human heart. Although the contribution of individual subtypes to the overall α_1-adrenoceptor density has been studied [e.g., in rat heart (Gross et al., 1988; Knowlton et al., 1993; Michel et al., 1994a) and rabbit heart (Endoh et al., 1992; Hattori et al., 1996)], little is known in this regard for the human heart at the protein level.

The functional role of α_1-adrenoceptors has been studied extensively in the nonhuman mammalian heart, and several review articles have been published on this topic (Endoh, 1991; Benfey, 1993; Terzic et al., 1993; Hein and Kobilka, 1997; Li et al., 1997a). Cardiac α_1-adrenoceptors can couple to numerous intracellular signal transduction responses—not only PLC and phospholipase D but also various ion currents, including L-type Ca^{2+} channels, the transient outward current I_{to}, the delayed rectifier K^+ current, and the acetylcholine-activated K^+ channel ($I_{K\ Ach}$); moreover, the Na^+/H^+ exchanger and Na^+,K^+-ATPase can be activated (Endoh, 1991). In transgenic mice overexpressing the wild-type (WT) hamster α_{1B}-adrenoceptor by more than 40-fold (Akhter et al., 1997a) or expressing a constitutively active mutant thereof (Milano et al., 1994b), myocardial diacylglycerol content was increased, indicating a tonic activation of the PLC pathway by cardiac α_1-adrenoceptors in vivo.

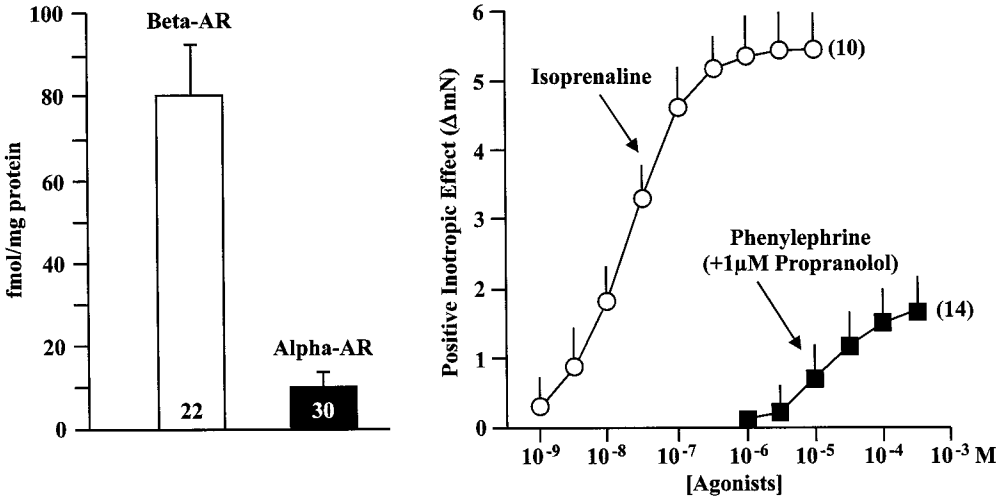

FIG. 2. Ventricular α_1- and β-adrenoceptors (ARs) in the nonfailing human heart. Left, number of β-adrenoceptors and α_1-adrenoceptors (in fmol/mg protein). Right, positive inotropic effect of isoprenaline (via β-adrenoceptor stimulation) and phenylephrine (in the presence of 1 μM propranolol via α_1-adrenoceptor stimulation) in isolated electrically driven ventricular trabeculae (in Δ mN). Numbers at the bottom of the columns (left) and in parentheses (right) indicate number of experiments. Data for α_1-adrenoceptor number are recalculated from Bristow et al. (1988), Böhm et al. (1988), and Steinfath et al. (1992a,b), and data for the positive inotropic effects of phenylephrine are recalculated from Böhm et al. (1988) and Steinfath et al. (1992b). Data for β-adrenoceptor number are recalculated from Bristow (1993), Brodde et al. (1998b), and Steinfath et al. (1992b), and data for the positive inotropic effects of isoprenaline are recalculated from Brodde et al. (1998b) and Steinfath et al. (1992b).

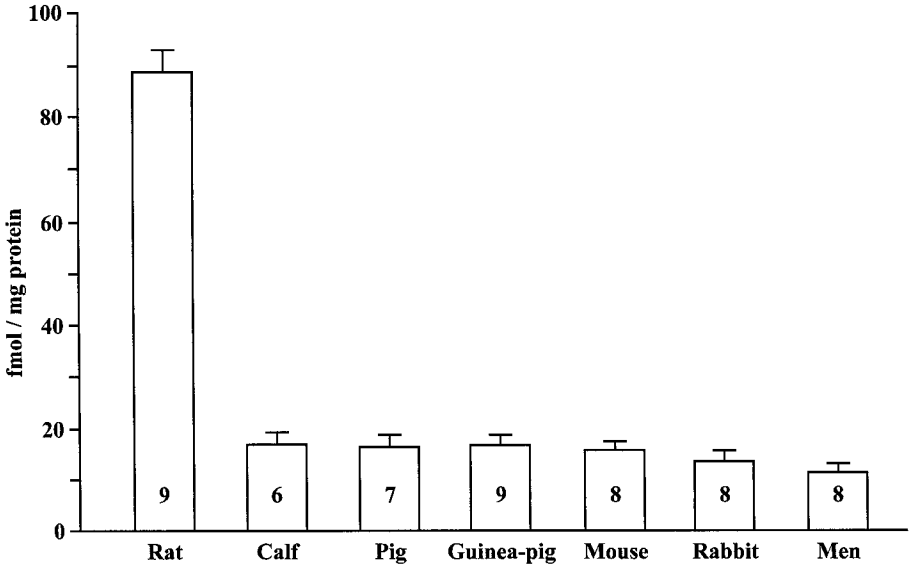

FIG. 3. Species dependence of cardiac α_1-adrenoceptor number, which was assessed by [^{3}H]prazosin binding in ventricular membranes. Numbers at the bottom of the columns indicate number of experiments. Data are recalculated from Steinfath et al. (1992a).

The relevance of this finding, however, is unclear because the physiological expression of α_1-adrenoceptors, largely representing the α_{1B}-adrenoceptor, is low in murine heart (Yang et al., 1998), and α_{1B}-adrenoceptor knockout mice do not show overt signs of an altered cardiovascular function (Cavalli et al., 1997). Stimulation of cardiac α_1-adrenoceptors does not lead to elevation of cellular cAMP content (Schümann et al., 1975; Brodde et al., 1978; Wagner and Brodde, 1978; Bogoyevitch et al., 1993) but if anything may even reduce cardiac cAMP content. This inhibition may relate to coupling to pertussis toxin (PTX)-sensitive G proteins of the G_i family rather than to activation of cAMP-degrading phosphodiesterases (Barrett et al., 1993). Accordingly, WT α_{1B}-adrenoceptor-overexpressing mice had mitigated inotropic responses to β-adrenergic stimulation, which were apparently due to α_{1B}-adrenoceptor coupling to PTX-sensitive G proteins resulting in adenylyl cyclase inhibition, and to up-regulation of the G protein-coupled receptor kinase 2 (GRK2, formerly named β-adrenergic receptor kinase; Akhter et al., 1997a). More distal signaling events after the stimulation of cardiac α_1-adrenoceptors involve the activation of protein kinase C (PKC; Clerk and Sugden, 1997), of which the α-, β_1,- β_2-, δ,- ϵ-, ι/λ-, and ζ-isoforms are present in human heart (Erdbrügger et al., 1997; Bowling et al., 1999), and the extracellular signal-regulated (Thorburn and Thorburn, 1994; Bogoyevitch et al., 1996; Post et al., 1996; Zechner et al., 1997) and p38 forms of the mitogen-activated protein kinases (Zechner et al., 1997; Clerk et al., 1998). The JNK forms of the mitogen-activated protein kinases, however, are not consistently activated on stimulation of cardiac α_1-adrenoceptors (Zechner et al., 1997). α_1-Adrenoceptor signal transduction results in enhanced inotropy under most conditions, but the net effect of the various signaling responses may also be a reduced force development secondary to PKC activation (Kissling et al., 1997; Peters et al., 1998). The mechanism of the positive inotropic effect induced by α_1-adrenoceptor stimulation is still a matter of debate: α_1-adrenoceptor stimulation causes formation of 1,4,5-inositoltrisphosphate and diacylglycerol, with the former mediating release of Ca^{2+} from intracellular stores, which might be involved in increases in force of contraction. In addition, α_1-adrenoceptor stimulation increases the Ca^{2+} sensitivity of myofilaments and the transsarcolemmal Ca^{2+} influx and causes intracellular alkalinization via activation of the Na^+/H^+ exchanger; it has been suggested that these effects are, at least in part, due to diacylglycerol-induced activation of PKC (Endoh, 1991; Terzic et al., 1993).

Apart from these short-term effects, extended stimulation of cardiac α_1-adrenoceptors may also cause the development of a hypertrophic phenotype. This has been studied in depth in cultured neonatal rat cardiomyocytes (Meidell et al., 1986; Lee et al., 1988; Waspe et al., 1990) but may also occur in cultured cardiomyocytes obtained from adult rats (Ikeda et al., 1991; Schlüter and Piper, 1992; Pinson et al., 1993) or even in rats in vivo (Zierhut and Zimmer, 1989). Whether these findings are applicable to other species is unclear because transgenic mice overexpressing WT α_{1B}-adrenoceptors by more than 40-fold did not develop signs of cardiac hypertrophy despite an 8-fold increase in ventricular mRNA for atrial natriuretic peptide (Akhter et al., 1997a). Only transgenic mice with cardiac overexpression of a constitutively active α_{1B}-adrenoceptor exhibited increased myocardial atrial natriuretic peptide mRNA and considerable cardiac hypertrophy (Milano et al., 1994b). Although it can be argued that mice have only a very low cardiac expression of α_1-adrenoceptor protein, this also is true for the human heart (Steinfath

et al., 1992a; Yang et al., 1998; see Fig. 3). Whether activation of α_1-adrenoceptors in the human heart promotes cardiac hypertrophy is unknown. Therefore, molecular pathways leading to α_1-adrenoceptor-stimulated cardiac hypertrophy in rats are only shortly summarized, and the reader is referred to several recent reviews on this topic for more details (Bogoyevitch and Sugden, 1996; Force et al., 1996; Page and Doubell, 1996; Olson and Molkentin, 1999; Sugden, 1999). α_1-Adrenoceptor-stimulated cardiac hypertrophy in rats occurs predominantly, if not exclusively, via α_{1A}-adrenoceptors (Knowlton et al., 1993; Autelitano and Woodcock, 1998), which is the subtype that dominates in the human heart, at least at the mRNA level (see above). These receptors act via the PTX-insensitive G protein G_q (LaMorte et al., 1994), which in turn leads to activation of PLC (Knowlton et al., 1993) and PKC (Shubeita et al., 1992; Karns et al., 1995; Bogoyevitch et al., 1996). Accordingly, transgenic cardiac overexpression of G_q (D'Angelo et al., 1997; Adams et al., 1998; Mende et al., 1998; Dorn et al., 1999) or transfection of cardiomyocytes with a constitutively active form of G_q (Adams et al., 1998) or PKCα, PKCβ, PKCϵ, or PKCζ causes cardiac hypertrophy (for reviews, see Bogoyevitch and Sugden, 1996; Simpson, 1999). On the other hand, cardiac overexpression of a construct to inhibit G_q function (a carboxyl-terminal peptide of the α-subunit of G_q) can inhibit overload-induced myocardial hypertrophy (Akhter et al., 1998). Distal to G_q activation, several signal-transducing molecules have been implicated in the development of α_1-adrenoceptor-stimulated cardiac hypertrophy, but the exact chain of events, and particularly the interaction between these mediators, remains a matter of debate (Bogoyevitch and Sugden, 1996; Force et al., 1996; Page and Doubell, 1996; Olson and Molkentin, 1999; Simpson, 1999; Sugden, 1999). Such mediators include the small GTP-binding proteins ras and rho, PKC, the extracellular signal-regulated and p38 members of the family of mitogen-activated protein kinases, and the protein phosphatase calcineurin. The latter is of particular interest for two reasons (for review, see Olson and Molkentin, 1999; Sugden, 1999): First, calcineurin may be an integrator and common mediator of several pathways leading to cardiac hypertrophy. Accordingly, calcineurin inhibition might prevent development of cardiac hypertrophy. This has in fact been shown in some animal models in vivo; however, several negative studies have also been published (for an overview, see Walsh, 1999). Second, inhibitors of calcineurin function (i.e., the immunosuppressant drugs cyclosporin A and tacrolimus, also known as FK506) are available that can be used to test hypotheses regarding the role of calcineurin in humans. However, the interpretation of the emerging human data is complicated by nephrotoxic effects of these drugs. Moreover, studies in humans are hampered by the fact that tissue material for investigation usually is available only from patients with late stages of disease processes, and some mediators relevant for cardiac hypertrophy induction may already have returned to baseline levels or even disappeared in those stages. Therefore, it remains unclear whether and how cardiac hypertrophy can develop secondary to myocardial α_1-adrenoceptor stimulation in humans.

The multitude of studies on the signal transduction of rat cardiac α_1-adrenoceptors is sharply contrasted by the very limited information that is available regarding the signal transduction of human cardiac α_1-adrenoceptors. Thus, noradrenaline stimulation (in the presence of propranolol) of human atrial appendages causes a breakdown of phosphatidylinositolbisphosphate as demonstrated by the rapid and time-dependent formation of inositol-1,3-bisphosphate, inositol-4-phosphate, inositol-1,4-bisphosphate, and inositol-1,4,5-trisphosphate (Bristow et al., 1988; Anderson et al., 1995). However, the extent of PLC activation in those studies was much less than that in rat atria studied under the same conditions, which is as to be expected based on the much smaller α_1-adrenoceptor density in the human heart (see Fig. 3).

It is generally assumed that α_1-adrenoceptors couple to their signal transduction machinery primarily via PTX-insensitive G proteins of the $G_{q/11}$ family (Bylund et al., 1994; Graham et al., 1996). Based on studies with rats, rabbits, and dogs, this also appears to be the case for many responses in the heart (Han et al., 1989; Steinberg et al., 1989; Del Balzo et al., 1990; Anyukhovsky et al., 1992; Braun and Walsh, 1993; Muntz et al., 1993; LaMorte et al., 1994; Liu et al., 1994; Sah et al., 1996; Hool et al., 1997), but coupling to PTX-sensitive G proteins may occur under some conditions (Steinberg et al., 1985; Han et al., 1989; Del Balzo et al., 1990; Keung and Karliner, 1990; Barrett et al., 1993; Anyukhovsky et al., 1994; Takeda et al., 1994) and has also been suggested in transgenic mice overexpressing WT α_{1B}-adrenoceptors (Akhter et al., 1997a). Moreover, it has recently been reported that α_1-adrenoceptors can also couple to a G protein that is larger than those of the $G_{q/11}$ family and designated G_h (Im et al., 1990; Im and Graham, 1990). In later studies, G_h was identified as identical with the enzyme tissue-type transglutaminase II (Nakaoka et al., 1994). The human cardiac α_1-adrenoceptor can also couple to G_h (Hwang et al., 1996). Although the functional consequences of such additional coupling are largely unknown, it is interesting that the extent of α_1-adrenoceptor coupling to G_h can be altered in human heart failure (see *Autonomic Responsiveness in Failing Human Heart*).

Most studies on the inotropic effects of α_1-adrenoceptor stimulation in human myocardium have used phenylephrine (in the presence of propranolol) as the agonist. As could be expected based on the small receptor number, the inotropic effects elicited by α_1-adrenoceptor stimulation were 15 to 35% of those elicited by β-adrenoceptor stimulation (Fig. 2) or receptor-independently by raising extracellular Ca^{2+}; such observations were made similarly in atrial (Schümann et al., 1978; Skomedal et al., 1985; Jahnel et al., 1992) and ventricular (Brückner et al., 1984; Aass et al., 1986; Böhm et

al., 1988; Jakob et al., 1988; Steinfath et al., 1992b) preparations. However, this may be partly due to the fact that phenylephrine is not a full agonist at any of the three human α_1-adrenoceptor subtypes (Taguchi et al., 1998). Accordingly, it was recently observed that the endogenous transmitter noradrenaline causes a more pronounced inotropic effect in isolated human myocardial strips than phenylephrine (Scholz et al., 1996; Skomedal et al., 1997).

When α_1-adrenoceptor-mediated positive inotropic effects were observed in the human heart, they were accompanied by no change in the action potential configuration (in ventricular myocardium; Jakob et al., 1988) or by a slight decrease in action potential duration (in atrial muscle; Jahnel et al., 1992), although in several animal species, stimulation of myocardial α_1-adrenoceptors prolongs the action potential duration (Brückner et al., 1985; for reviews, see Endoh, 1991; Endoh et al., 1991; Terzic et al., 1993; Li et al., 1997a). Based on these observations, it could be expected that the endogenous catecholamines noradrenaline and adrenaline exert their positive inotropic effects mainly via β- rather than α_1-adrenoceptor stimulation. Indeed, two studies with noradrenaline or adrenaline as the agonists were unable to detect inhibition of inotropic effects by the nonselective α-adrenoceptor antagonist phentolamine or the selective α_1-adrenoceptor antagonist prazosin (Jakob et al., 1988; Jahnel et al., 1992). In contrast, another study has reported that the contributions of α_1- and β-adrenoceptors to the inotropic effects of noradrenaline are similar (Skomedal et al., 1997). Responses to endogenous noradrenaline (released by tyramine administration) were reported to occur via α_1- and β-adrenoceptors to 14 and 86%, respectively (Borthne et al., 1995). These divergent results may in part be explained by the use of different tissue sources. Thus, the negative studies have used atrial or ventricular preparations from patients with only mild to moderate heart failure (New York Heart Association functional class II/III), whereas the positive study has used ventricular tissue from patients with severe heart failure (transplant recipients). Because a decrease in β-adrenoceptor-mediated positive inotropic effects in advanced heart failure is well documented (Brodde, 1991), a relative enhancement of the α-adrenergic effects could be possible under these conditions (but see *Autonomic Responsiveness in the Failing Human Heart. A. α_1-Adrenoceptors*).

An analysis of the possible contributions of α_1-adrenoceptors to positive inotropic effects of noradrenaline in vivo is difficult because a possible direct effect on the cardiomyocytes may be mimicked and/or concealed by the effects of α_1-adrenoceptor stimulation in the systemic and/or coronary vasculature. One possible experimental approach to this problem is enhancement of endogenous noradrenaline release by tyramine administration. Although i.v. infusion of tyramine caused positive inotropic effects in young healthy volunteers, this was not inhibited by concomitant α_1-adrenoceptor blockade with doxazosin (Schäfers et al., 1997). Another approach is the intracoronary injection or infusion of α_1-adrenoceptor agonists, which at least prevents the problem of afterload elevations. Direct intracoronary infusion of phenylephrine enhanced left ventricular peak (+)dP/dt in a dose-dependent manner; part of this response was sensitive to the α-adrenoceptor antagonist phentolamine, indicating that it was mediated by an α-adrenoceptor (Landzberg et al., 1991). On the other hand, phentolamine alone did not modify cardiac contractility in that study, indicating that α-adrenoceptors do not contribute to the maintenance of basal left ventricular contractile state in humans at rest. Studies with i.v. administration of the α_1-adrenoceptor agonist methoxamine in comparison with angiotensin II have also suggested that a small but detectable component of the inotropic response may occur via α_1-adrenoceptors in humans in vivo (Curiel et al., 1989).

Taken together, these data clearly indicate that stimulation of α_1-adrenoceptors can cause positive inotropic effects in the isolated human heart. Although α-adrenoceptor-mediated inotropic effects in rat ventricle appear to occur primarily via α_{1B}-adrenoceptors (Michel et al., 1994b), little is known about the α_1-adrenoceptor subtype causing positive inotropic effects in humans. Although α_1-adrenoceptors may also cause positive inotropic effects in vivo, this may be of limited physiological relevance because the endogenous agonist noradrenaline acts only to a minor degree, if any, via cardiac α_1-adrenoceptors. However, present data are insufficient to exclude that α_1-adrenoceptors participate in the regulation of cardiac force development specifically in settings such as congestive heart failure (see *Autonomic Responsiveness in Failing Human Heart*) or on blockade of β-adrenoceptors. Thus, at least in rat hearts, it has been observed that in vivo treatment with the β-adrenoceptor antagonist propranolol increases α_1-adrenoceptor number (Mügge et al., 1985; Steinkraus et al., 1989) and enhances the positive inotropic effects of α_1-adrenoceptor stimulation in vitro (Li et al., 1997b).

B. α_2-Adrenoceptors

Three human α_2-adrenoceptor subtypes exist (α_{2A}, α_{2B}, and α_{2C}; Fig. 1; Bylund et al., 1994); their pharmacological characteristics are shown in Table 2. They are encoded by distinct genes that are located on the human chromosomes 10q23-25, 2, and 4, respectively. The ortholog of the human α_{2A}-adrenoceptor in some species exhibits a markedly different pharmacological recognition profile despite only minor differences in its deduced amino acid sequence and is referred to as the α_{2D}-adrenoceptor (Bylund et al., 1994). Several studies have investigated the presence of α_2-adrenoceptor subtypes in the human heart at the mRNA level using RNase protection assays or RT-PCR. RT-PCR studies have reported the presence of all three subtypes in endocardium

TABLE 2
Pharmacological characterization of α_2-adrenoceptor subtypes

	α_{2A}[a]	α_{2B}	α_{2C}
Potency order		adrenaline > noradrenaline	
Selective agonists	Oxymetazoline[b]		
Selective antagonists	BRL 44408 (8.0)	ARC 239 (8.0) Prazosin (7.5) Imiloxan (7.3)	ARC 239 (8.0) Prazosin (7.5)

Antagonist affinities (number in parentheses) are expressed as approximate $-\log K_i$ values. Adapted from Alexander and Peters (1999).
[a] α_{2A}-adrenoceptors from rats, mice, and cows have a ≈20-fold lower affinity for yohimbine, rauwolscine, and oxymetazoline than do their human orthologs.
[b] Reduced efficacy agonist.

at the qualitative level but have failed to detect α_{2B}-adrenoceptor mRNA in left ventricular epicardium despite the presence of the other two subtypes in this tissue (Eason and Liggett, 1993). In contrast, RNase protection assays have not confirmed the presence of α_{2A}-adrenoceptor mRNA in the human heart but have detected mRNA for α_{2B}-adrenoceptors and with an even greater abundance for α_{2C}-adrenoceptors (Perälä et al., 1992; Berkowitz et al., 1994). However, it should be noted that in a direct comparison, even the abundance of mRNA for the α_{2C}-adrenoceptors was 30-fold less than that for the α_{1A}-adrenoceptor (Berkowitz et al., 1994). In the fetal human heart, mRNA for α_{2A}- or α_{2C}-adrenoceptors has not been detected (Perälä et al., 1992). In light of this small α_2-adrenoceptor subtype mRNA abundance, it is not surprising that we have not been successful in demonstrating α_2-adrenoceptors in human heart at the protein level through radioligand binding studies (OEB and MCM, unpublished observations) and are not aware of any other studies to this effect.

Functional studies on human cardiac α_2-adrenoceptors have focused on presynaptic inhibition of noradrenaline release. Thus, α_2-adrenoceptor-mediated prejunctional inhibition has repeatedly been demonstrated in isolated human atrial appendages (Rump et al., 1995a,b; Likungu et al., 1996). The prejunctional inhibitory receptor in the human atrium has originally been classified as an α_{2C}-adrenoceptor (Rump et al., 1995a). This contrasts evidence from a variety of tissues and species, where the prejunctional α_2-adrenoceptor belongs to the α_{2A} subtype or its species ortholog α_{2D} (Trendelenburg et al., 1997). However, this discrepancy between human heart and other tissues should not be overinterpreted for two reasons. First, the pharmacological tools used at the time did not allow a very good discrimination between α_{2A}- and α_{2C}-adrenoceptors. Second, the same authors, using the same tools, have also classified the prejunctional receptor in human kidney as belonging to the α_{2C} subtype (Trendelenburg et al., 1994) but have reclassified it as α_{2A} based on newer tools (Trendelenburg et al., 1997).

The discrepancy between pharmacological classification of prejunctional α_2-adrenoceptors in the human heart as α_{2A} and the apparent absence of corresponding mRNA (see above) is not surprising because the perikarya of the cardiac sympathetic neurons reside in the sympathetic chain.

To test the functional relevance of these in vitro findings for the in vivo setting, studies with intracoronary infusion of the α-adrenoceptor antagonist phentolamine have been performed (Parker et al., 1995). In these studies, intracoronary phentolamine infusion did not modify catecholamine spillover in subjects with normal left ventricular function but enhanced it in patients with congestive heart failure. Because a catecholamine release-enhancing effect of α-adrenoceptor antagonists depends on the presence of a tonically active catecholamine release and because an enhanced sympathetic drive in congestive heart failure is well documented (Packer, 1992), these data suggest that prejunctional α_2-adrenoceptors play a functional noradrenaline release-inhibiting role in the human heart that becomes evident under conditions of enhanced sympathetic activity.

C. β-Adrenoceptors

Three different β-adrenoceptor subtypes have been cloned so far and identified pharmacologically: β_1, β_2, and β_3 (Fig. 1; Bylund et al., 1994). These subtypes are encoded by three distinct genes that are located on human chromosomes 10q24-26, 5q31-32, and 8p11-12. The molecular structure of the β_3-adrenoceptor and its gene differ in various ways from those of the β_1- and β_2-adrenoceptors: Thus, the human β_3-adrenoceptor gene has introns, whereas the β_1- and β_2-adrenoceptor genes do not (Granneman et al., 1993; Van Spronsen et al., 1993). Moreover, the human β_3-adrenoceptor lacks sites for phosphorylation by cAMP-dependent protein kinase (PKA) and GRK2 in its carboxyl terminus that are readily found in the β_1- and β_2-adrenoceptors (Hausdorff et al., 1990; Emorine et al., 1991; Strosberg, 1997a). Finally, there are marked species differences between rodent and human β_3-adrenoceptors with respect to expression in white versus brown adipose tissue and the sensitivity to stimulation by certain β_3-adrenoceptor-selective agonists (Strosberg, 1997a), whereas such species differences obviously do not exist for β_1- and β_2-adrenoceptors (Bylund et al., 1994). Recent pharmacological studies, mainly in human and rat cardiac tissue, have proposed the existence of a fourth β-adrenoceptor (see below), but this subtype has not been cloned so far. The pharmacological characteristics of β-adrenoceptor subtypes are shown in Table 3.

1. β_1- and β_2-Adrenoceptors. In the human heart, the existence of β_1- and β_2-adrenoceptors has been demonstrated at the mRNA level with RT-PCR and RNase protection assay (Bristow et al., 1993; Ungerer et al.,

TABLE 3
Pharmacological characterization of β-adrenoceptor subtypes

	β_1	β_2	β_3	β_4 (?)
Potency order	ISO > E = NE	ISO > E > NE	ISO = NE > E	?
Selective agonists	NE[a] Xamoterol[a,b] RO 363 (nM)[a,b]	Terbutaline Salbutamol Procaterol Fenoterol Zinterol	CGP 12177[c] CL 316243[d] BRL 37344[d]	CGP 12177[c] RO 363 (μM)
Selective antagonists	CGP 20712A (8.5–9.3) Betaxol (8.5) Atenolol (7.6) Bisoprolol (8.1–8.8)	ICI 118,551 (8.3–9.2)	Bupranolol[c] (6.9–7.3) SR 59230A (7.5–8.8)	Bupranolol[c] (6.4–7.3)

ISO, isoprenaline; NE, noradrenaline; E, adrenaline. Drug affinities (numbers in parentheses) are expressed as $-\log K_i$ or $-\log K_B$ values. Adapted from Alexander and Peters (1999), Bylund et al. (1998), Brodde (1997), Kaumann and Molenaar (1997), Manara et al. (1996), Molenaar et al. (1997).
[a] Selective relative to β_2-adrenoceptors.
[b] In some tissues, partial agonists.
[c] Antagonists with high affinity at β_1- and β_2-adrenoceptors.
[d] Have higher intrinsic activity at rodent β_3-adrenoceptors than at human β_3-adrenoceptors.

1993; Engelhardt et al., 1996; Ihl-Vahl et al., 1996), at the protein level with radioligand binding, and in functional studies both in vitro and in vivo (for reviews, see Jones et al., 1989; Bristow et al., 1990; Brodde, 1991; Bristow, 1993; Harding et al., 1994; Brodde et al., 1995a; Kaumann and Molenaar, 1997). The coexistence of β_1- or β_2-adrenoceptors has also been demonstrated on isolated human ventricular cardiomyocytes (Del Monte et al., 1993).

Both β_1- and β_2-adrenoceptors in the human heart couple to G_s to activate adenylyl cyclase, and stimulation of both receptor subtypes increases the intracellular level of cAMP. This subsequently leads to activation of PKA, which phosphorylates several sarcolemmal proteins, including L-type Ca^{2+} channels and phospholamban (Walsh and Van Patten, 1994; Kaumann and Molenaar, 1997). Phosphorylation of L-type Ca^{2+} channels promotes Ca^{2+} influx and thus enhances contraction; phosphorylation of phospholamban may be involved in enhanced diastolic relaxation by increasing Ca^{2+} uptake into the sarcoplasmic reticulum. Although G_s can also directly activate L-type Ca^{2+} channels in nonhuman mammalian cardiomyocytes (Clapham, 1994; Schneider et al., 1997), it is not known whether this also occurs in the human heart. Moreover, in human atrial and ventricular myocardium, both β_1-and β_2-adrenoceptor stimulation enhances myocardial relaxation (for references, see Kaumann and Molenaar, 1997); in addition, both β_1- and β_2-adrenoceptor stimulation causes phosphorylation of phospholamban by PKA (Kaumann et al., 1996, 1999).

Lakatta and colleagues proposed that in adult rat ventricular cardiomyocytes, β_2-adrenoceptors can also couple to G_i (Xiao and Lakatta, 1993; Xiao et al., 1994, 1995). Thus, in rat cardiomyocytes, the β_2-adrenoceptor agonist zinterol increased the $[Ca^{2+}]_i$ transient amplitude and caused positive inotropic effects that could be enhanced by pretreatment of the cells with PTX; no such potentiation by PTX treatment was observed for noradrenaline acting predominantly at β_1-adrenoceptors. In addition, β_2-adrenoceptor stimulation (in contrast to β_1-adrenoceptor stimulation) did not correlate well with increases in intracellular cAMP, did not lead to phosphorylation of phospholamban in these cells, and did not hasten relaxation; after PTX treatment, however, relaxation was increased. Comparable data were recently obtained in murine ventricular cardiomyocytes where the β_2-adrenoceptor agonist zinterol failed to increase force of contraction or $[Ca^{2+}]_i$ transient amplitude; however, after the treatment of the cardiomyocytes with PTX, zinterol, via β_2-adrenoceptor stimulation, increased both parameters significantly (Xiao et al., 1999). This PTX treatment-unmasked β_2-adrenoceptor response was mediated by a cAMP-dependent mechanism because it could be completely blocked by the inhibitory cAMP analog Rp-cAMPs. Thus, in the mouse heart, β_2-adrenoceptors obviously couple to G_s and G_i. On the other hand, Laflamme and Becker (1998) recently failed to demonstrate any effect of β_2-adrenoceptor stimulation on Ca^{2+} homeostasis in isolated adult rat cardiomyocytes; they also could not find any effect of PTX treatment. Moreover, Skerberdis et al. (1997) demonstrated that in frog and rat ventricular cardiomyocytes, stimulation of β_2-adrenoceptors by zinterol increased L-type Ca^{2+} current in a totally cAMP-dependent manner; when cAMP-dependent phosphorylation was blocked by a highly selective PKA inhibitor, effects of zinterol on L-type Ca^{2+} current were completely blocked. Finally, Yatani et al. (1999) recently reported that in CHW cells stably expressing cardiac Ca^{2+} channels together with either β_1- or β_2-adrenoceptors, isoprenaline was more efficacious in activation of the Ca^{2+} channels via β_1-adrenoceptor stimulation versus β_2-adrenoceptor stimulation. The effects of both β_1- and β_2-adrenoceptor stimulation on Ca^{2+} channel activation were not affected by PTX treatment of the cells, indicating that the subtype-selective coupling of β_1- and β_2-adrenoceptors to the Ca^{2+} channels is not due to differential coupling to a PTX-sensitive G protein. Possibly, there are kinetic differences between β_1- and β_2-adrenoceptor-mediated activation of L-type Ca^{2+} channels (Schröder and Herzig, 1999): although β_1-adrenoceptor stimulation reduced

mean closed time of the channel, β_2-adrenoceptor stimulation (with zinterol) did not but did reduce the "relative abundance of very short-lived bursts". Whether human cardiac β_1- and/or β_2-adrenoceptors might also couple to G_i is unknown.

In the human heart, the β_1/β_2-adrenoceptor ratio is about 60 to 70%:40 to 30% in the atria and about 70 to 80%:30 to 20% in the ventricles (Brodde, 1991). Interestingly, Rodefeld et al. (1996) have recently shown that in human sinoatrial nodes, β-adrenoceptor densities were about 3-fold higher than that in the adjacent atrial myocardium; although the β_1-adrenoceptor subtype predominates, the β_2-adrenoceptor density was about 2.5-fold higher in the sinoatrial node than in the right atrial myocardium.

Despite the fact that β_1-adrenoceptors predominate in human myocardium, the functional responses mediated by β_1- and β_2-adrenoceptors are not necessarily different. This may be due to the fact that human cardiac β_2-adrenoceptors are more effectively coupled to adenylyl cyclase than are β_1-adrenoceptors. This has been demonstrated in human right atrium (Brodde et al., 1984; Gille et al., 1985), human left ventricle (Bristow et al., 1989; Kaumann et al., 1989), and, finally, cells transfected with human β_1- and β_2-adrenoceptors either separately (Green et al., 1992) or together (Levy et al., 1993). The more effective coupling of β_2-adrenoceptors to adenylyl cyclase might explain why isoprenaline and adrenaline cause nearly identical increases in force of contraction via β_1- or β_2-adrenoceptor stimulation in vitro on isolated human right atrium (Kaumann et al., 1989; Motomura et al., 1990b) despite the predominance of β_1-adrenoceptors. On the other hand, in isolated ventricular preparations, only β_1-adrenoceptor stimulation causes maximal increases in force of contraction, whereas β_2-adrenoceptor stimulation causes only submaximal increases in force of contraction (Kaumann et al., 1989; Motomura et al., 1990b). Noradrenaline, however, increases contractility predominantly (if not exclusively) via β_1-adrenoceptor stimulation in isolated atrial and ventricular preparations in vitro (Kaumann et al., 1989; Motomura et al., 1990b).

In vivo studies have confirmed that both β_1- and β_2-adrenoceptors are involved in positive inotropic and chronotropic effects. Several groups have shown that isoprenaline infusion-induced increases in heart rate in humans are mediated by β_1- and β_2-adrenoceptor stimulation to about the same degree (McDevitt, 1989; Brodde, 1991), possibly due to the fact that in human sinoatrial node, β_2-adrenoceptor density is quite high (see above). There had been a long controversy over whether these isoprenaline effects are direct effects on cardiac β_2-adrenoceptors or indirect effects caused by a reflex mechanism resulting from vasodilatation (reflex withdrawal of cardiac vagal tone). Three sets of data, however, support the view that these positive chronotropic effects are mediated via direct stimulation of cardiac β_2-adrenoceptors. First, Hall et al. (1989) have shown that intracoronary injection of the β_2-adrenoceptor agonist salbutamol causes increases in heart rate that are not affected by the β_1-adrenoceptor antagonist practolol but are blocked by the nonselective β-adrenoceptor antagonist propranolol. Second, in heart transplant recipients, isoprenaline increases heart rate under conditions where it solely acts via β_2-adrenoceptors (i.e., in the presence of the highly selective β_1-adrenoceptor antagonist bisoprolol; Hakim et al., 1997); this effect cannot be due to any reflex mechanisms because the transplanted human heart is a denervated organ. Third, Leenen et al. (1995) recently observed in heart transplant recipients that exercise-induced heart rate increases (normally mediated solely by β_1-adrenoceptor stimulation, see below) were more effectively blocked by the nonselective β-blocker nadolol than by the β_1-selective blocker atenolol, indicating that in these patients, stimulation of cardiac β_2-adrenoceptors plays an important role in exercise-induced tachycardia. On the other hand, the positive inotropic effect of isoprenaline in vivo is brought about predominantly through β_1-adrenoceptor stimulation (Wellstein et al., 1988; Schäfers et al., 1994).

In contrast to isoprenaline, noradrenaline evokes its in vivo effects (very similar to its in vitro effects; see above) predominantly via cardiac β_1-adrenoceptor stimulation: exercise-induced tachycardia (which is believed to be due to neuronally released noradrenaline) is mediated solely through β_1-adrenoceptor stimulation (McDevitt, 1989; Brodde, 1991). Similarly, recent studies in healthy volunteers demonstrated that infusion of noradrenaline (i.e., exogenous application) and release of endogenous noradrenaline (by infusion of tyramine) increases contractility predominantly via stimulation of β_1-adrenoceptors (Schäfers et al., 1997). Moreover, in heart transplant recipients (where parasympathetic buffering is absent; see above), Leenen et al. (1998) recently demonstrated that noradrenaline infusion caused positive inotropic and chronotropic effects that were mediated nearly exclusively via β_1-adrenoceptor stimulation.

Although adrenaline increases contractility of isolated electrically driven right atrial preparations in vitro via stimulation of β_1- and β_2-adrenoceptors to about the same degree (Kaumann et al., 1989; Motomura et al., 1990b), it induces tachycardia in healthy volunteers in vivo nearly exclusively through β_2-adrenoceptor stimulation (Brown et al., 1983; Leenen et al., 1988; Daul et al., 1995; Fig. 4). On the other hand, its in vivo effect on contractility in healthy volunteers is mediated by β_1- (to a larger extent) and β_2-adrenoceptor (to a minor extent) stimulation (Leenen et al., 1988); the contribution of β_2-adrenoceptor stimulation appears to be increased in patients with hypertension and in heart transplant recipients (Leenen et al., 1998).

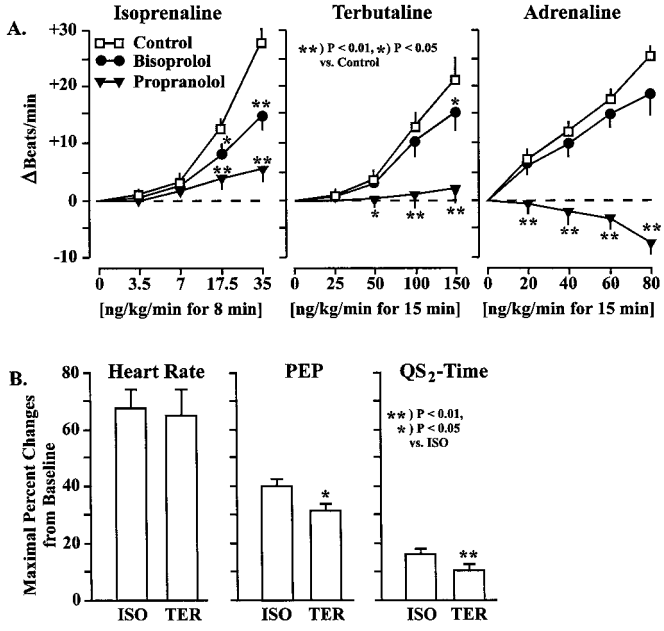

FIG. 4. A, effect of the β_1-adrenoceptor antagonist bisoprolol (15 mg p.o. 2 h before agonist infusion) or of propranolol (5 mg i.v. 45 min before agonist infusion) on isoprenaline (left), terbutaline (middle), and adrenaline (right) infusion-induced increases in heart rate in eight healthy male volunteers. Ordinate, increase in heart rate (in Δ bpm). Abscissa, dose of agonists (in ng/kg/min). Values are means ± S.E. modified from Daul et al. (1995). B, isoprenaline (ISO) and terbutaline (TER) infusion-induced maximal increases in heart rate and maximal shortening of the systolic time intervals (as measure of inotropism) pre-ejection period (PEP) and heart rate-corrected duration of electromechanical systole (QS_2) in seven healthy male volunteers. Ordinate, maximal increase in heart rate (left) and shortening of PEP (middle) and QS_2 time (right) expressed as maximal percent changes from baseline. Values are means ± S.E. recalculated from Schäfers et al. (1994).

Finally, several groups have demonstrated directly β_2-adrenoceptor-mediated positive inotropic and chronotropic effects in humans in vivo using β_2-adrenoceptor agonists such as terbutaline (Fig. 4) or salbutamol (Strauss et al., 1986; Levine and Leenen, 1989; Schäfers et al., 1994; Poller et al., 1998; Newton et al., 1999). Interestingly, the positive inotropic effects of high doses of terbutaline and salbutamol were slightly antagonized by β_1-selective blockers such as bisoprolol or atenolol. In addition, Newton et al. (1999) noticed that during intracoronary artery infusion of salbutamol, cardiac noradrenaline spillover was markedly enhanced. These data are in favor of the idea that the activation of prejunctional β_2-adrenoceptors (which have been demonstrated to exist in human heart; Hill et al., 1987; Rump et al., 1994) leading to enhanced noradrenaline release might contribute to the cardiac effects of β_2-adrenoceptor agonists, at least at higher doses.

2. Is There a Third (or Fourth) β-Adrenoceptor Subtype Present in Human Heart? During the past few years, evidence has accumulated that in addition to β_1- and β_2-adrenoceptors, a third or fourth (or both) β-adrenoceptor might exist in the human heart. The existence of a third β-adrenoceptor had been originally suggested based on the findings that in guinea pig and cat hearts, "nonconventional" β-adrenoceptor antagonists with partial agonistic activity (e.g., pindolol and congeners) exhibited stimulatory properties in concentrations exceeding those required for β-adrenoceptor blockade (Kaumann, 1989). The site mediating these effects could be the cloned β_3-adrenoceptor (discovered by Strosberg and his group [Emorine et al., 1989]) and/or "the putative β_4-adrenoceptor" (described by Kaumann, 1996).

Whether the cloned β_3-adrenoceptor exists in the human heart is still a matter of debate. Berkowitz et al. (1995) and Krief et al. (1993) did not detect β_3-adrenoceptor mRNA in human left ventricular tissue by RNase protection assay or RT-PCR, respectively; however, some β_3-adrenoceptor mRNA was found in left atria but only in fatty atrial samples, and this may reflect periatrial fat rather than atrial cardiomyocytes (Strosberg, 1997a). On the other hand, Gauthier et al. (1996) recently found mRNA of β_3-adrenoceptors in endomyocardial biopsy samples from right intraventricular septum of cardiac transplant recipients by RT-PCR.

Functional studies on possible β_3-adrenoceptor-mediated effects in the human heart have also remained inconclusive. Thus, Gauthier et al. (1996) observed in ventricular endomyocardial biopsy samples of heart transplant recipients that in the presence β_1- and β_2-adrenoceptor-blocking concentrations of nadolol, isoprenaline (at concentrations >1 μM, i.e., supramaximal concentrations in regard to positive inotropic effects) caused negative inotropic effects. Interestingly, these negative inotropic effects were also obtained with the β_3-adrenoceptor agonists BRL 37344, SR 58611, and CL 316243, although these "rodent" β_3-adrenoceptor agonists (cf. Table 3) have been described to be quite ineffective at the human β_3-adrenoceptor (Strosberg, 1997a). The effects of the β_3 agonists could be inhibited by bupranolol (an antagonist at β_1-, β_2-, and β_3-adrenoceptors; Strosberg 1997a) but not by the β_1-adrenoceptor antagonist metoprolol or the β_1- and β_2-adrenoceptor antagonist nadolol. Moreover, the negative inotropic effect of BRL 37344 was sensitive to PTX treatment, indicating that the β_3-adrenoceptor in human ventricular myocardium may couple to a G_i protein, as has been shown for the β_3-adrenoceptors in adipocyte tissue (Chaudry et al., 1994). In a subsequent study, these authors also demonstrated that in endomyocardial biopsy samples from right intraventricular septum of cardiac transplant patients, the negative inotropic effect of BRL 37344 and noradrenaline (in the presence of prazosin to block α_1-adrenoceptors and nadolol to block β_1- and β_2-adrenoceptors) is mediated through stimulation of nitric oxide synthase (NOS) III because it was attenuated by methylene blue, an inhibitor of NO-dependent activation of soluble guanylyl cyclase and by the NOS inhibitors, N^G-monomethyl-L -arginine (L-NMMA) and N^G-nitro-L-arginine methyl ester (L-NAME) (Gauthier et al., 1998). An excess of L-arginine could reverse the effects of the NOS inhibitors. On the other hand, Harding (1997) failed to demonstrate any effect of β_3-adrenoceptor stimulation in human ventricular

myocytes. Similarly, preliminary experiments by Kaumann and Molenaar (1997) and Molenaar et al. (1997a) suggest that in neither human right ventricular trabeculae nor human right atrial preparations did BRL 37344 cause a negative inotropic effect. Taken together, the presence and function of β_3-adrenoceptors in the human heart are still uncertain, and further studies must clarify whether β_3-adrenoceptors are involved in the regulation of human cardiac function.

Despite the controversy regarding the presence and function of β_3-adrenoceptors in the human heart, evidence has accumulated in the past few years that a β-adrenoceptor distinct from β_1- and β_2-adrenoceptors (and possibly also from β_3-adrenoceptors) can mediate increases in rate of beating and in force of contraction in rat and human heart. The characterization of this receptor has mainly relied on CGP 12177 as the agonist, which is also an antagonist at β_1- and β_2-adrenoceptors and an agonist at β_3-adrenoceptors (Arch and Kaumann, 1993). Thus, in isolated, electrically stimulated human right atrial appendages (−)-CGP 12177 [in the presence of 200 nM (−)-propranolol to block β_1- and β_2-adrenoceptors] has been shown to increase force of contraction (Kaumann, 1996). Moreover, (±)-CGP 12177 caused positive chronotropic effects in the pithed rat that were not antagonized by conventional β_1- or β_2-adrenoceptor-selective antagonists (Malinowska and Schlicker, 1996). Subsequently, it has been shown that this receptor is present in rat, mouse, ferret, and human heart (Molenaar et al., 1997a; Lowe et al., 1998) and can be labeled with the radioligand (−)-[^{3}H]CGP 12177 (Sarsero et al., 1998). In rat atria, (−)-CGP 12177 activates PKA; moreover, the phosphodiesterase inhibitor isobutylmethylxanthine potentiated the positive inotropic and chronotropic effects of (−)-CGP 12177 (Kaumann and Lynham, 1997). These data indicate that this receptor couples to the G_s protein/adenylyl cyclase system (in sharp contrast to the putatively G_i-coupled cardiac β_3-adrenoceptor proposed by Gauthier et al., 1996, 1998, see above).

Although the putative third cardiac β-adrenoceptor resembles the β_3-adrenoceptor, the following arguments are in favor of the idea that the third cardiac β-adrenoceptor (designated "the putative β_4-adrenoceptor") is different from the β_3-adrenoceptor (Kaumann and Molenaar, 1997; Molenaar et al., 1997a): First, the putative β_4-adrenoceptor was potently activated by (−)-CGP 12177 but not by selective β_3-adrenoceptor agonists such as BRL 37344 or CL 316243 (Kaumann and Molenaar, 1996). Second, the effects of (−)-CGP 12177 were antagonized by bupranolol (although with an affinity about 10–30 times lower than that at β_1- and β_2-adrenoceptors) but not by the selective β_3-adrenoceptor antagonist SR59230A (Manara et al., 1996), which is in contrast to its effect on the cloned human β_3-adrenoceptor or to the relaxant effects of CGP 12177 on the rat colonic β_3-adrenoceptor (Molenaar et al., 1997b). Third, specific binding of (−)-[^{3}H]CGP 12177 to the putative β_4-adrenoceptor in rat atrium was inhibited by the agonist (−)-CGP 12177 and the antagonist bupranolol but not by the selective β_3 agonists BRL 37344, SR58611A, and CL 316243 or the selective β_3 antagonist SR 59230A (Sarsero et al., 1998). Recently, two groups (Susulic et al., 1995; Grujic et al., 1997) succeeded in targeted inactivation of the β_3-adrenoceptor gene in the mouse. In these β_3-adrenoceptor knockout mice, (−)-CGP 12177 increased force of contraction of left atria and rate of beating of spontaneously beating right atria, and these effects were not blocked by propranolol but were potently antagonized by bupranolol and were not significantly different from those obtained in WT mice (Kaumann et al., 1998). Moreover, binding of (−)-[^{3}H]CGP 12177 (in the presence of 500 nM propranolol to block β_1- and β_2-adrenoceptors) revealed nearly identical numbers of binding sites in WT and β_3-adrenoceptor knockout mice (Kaumann et al., 1998), thus strongly supporting the view that the putative cardiac β_4- and β_3-adrenoceptors are different receptors. However, this can only be decided definitively when the β_4-adrenoceptor has been cloned.

Interestingly, using the same model of β_3-adrenoceptor knockout mice, Preitner et al. (1998) found that in brown adipose tissues (the major source of β_3-adrenoceptors; Strosberg, 1997a) (±)-CGP 12177 evoked a full metabolic response (assessed as O_2 uptake) comparable to that obtained in WT mice, whereas the effects of the β_3-adrenoceptor agonist CL 316243 were abolished. Thus, it appears that in rodent brown adipose tissue, β_1-, β_2-, and β_3-adrenoceptors and the putative β_4-adrenoceptor might coexist. Similarly, it is still a matter of controversy whether in human adipocyte tissues, β_3- or another β-adrenoceptor subtype with similarities to the putative β_4-adrenoceptor is the major subtype mediating metabolic effects (see Galitzky et al., 1998; and the reply by Strosberg et al., 1998). In this context, it is interesting to note that for the in vivo demonstration of β_3-adrenoceptor-mediated lipolytic effects in humans, CGP 12177 has been predominantly used as the agonist (Lönnqvist et al., 1993; Enocksson et al., 1995), and as discussed above, CGP 12177 is a potent agonist at the putative β_4-adrenoceptor.

One key experiment, however, is necessary before the putative β_4-adrenoceptor can be called a β-adrenoceptor: According to all classification criteria, a β-adrenoceptor is classified as a receptor that is activated by isoprenaline and physiological concentrations of the endogenous catecholamines noradrenaline and adrenaline. An effect induced by physiological concentrations of noradrenaline and adrenaline on the putative β_4-adrenoceptor has not been demonstrated so far; therefore, it remains uncertain whether the putative β_4-adrenoceptor indeed represents a novel adrenoceptor subtype or whether in the human heart CGP 12177 acts via a distinct "cardiac CGP 12177 site". In this context, it is interesting to note that studies in CHW cells expressing β_1-adrenoceptors show

that (±)-CGP 12177 can activate β_1-adrenoceptors and that its level of agonist activity is related to the level of β_1-adrenoceptor expression (Pak and Fishman, 1996). Moreover, Konkar et al. (1999) recently showed that CGP 12177 activated human β_1-adrenoceptors expressed in Chinese hamster ovary cells; this effect was significantly more resistant to blockade by bupranolol (>10-fold) than were the effects of the catecholamines isoprenaline and noradrenaline. On the other hand, agonist effects of CGP 12177 and of both catecholamines at the human β_3-adrenoceptor expressed in Chinese hamster ovary cells were blocked by bupranolol with equivalent potencies. These results suggest that CGP 12177 labels a site/state of the β_1-adrenoceptor that is different from the site/state labeled by catecholamines and that is relatively resistant to β-adrenoceptor blockade by bupranolol. Finally, in a rat model of cardiac failure, Kompa and Summers (1999) recently observed that changes in putative β_4-adrenoceptors followed a very similar pattern as those observed for β_1-adrenoceptors. Thus, it cannot be excluded that the putative β_4-adrenoceptor might be a "propranolol/bupranolol-insensitive" form of the β_1-adrenoceptor. Studies in β_1-adrenoceptor knockout mice could help to resolve this problem.

Such genetic knockout mice with targeted disruption of the genes for β_1-adrenoceptors (Rohrer et al., 1996), β_2-adrenoceptors (Chruscinski et al., 1999), and β_1- and β_2-adrenoceptors (Rohrer et al., 1999) have recently been created. These knockout mice could be very valuable experimental tools in which to study the role of β-adrenoceptor subtypes in the heart. Rohrer et al. (1996) were the first to describe the β_1-adrenoceptor knockout mice and the resultant phenotype. The majority of the β_1-adrenoceptor null mutants died prenatally, indicating that β_1-adrenoceptors play an important role in mouse development in utero. In the mouse heart, similar to the human heart, β-adrenoceptor subtypes coexist in a β_1/β_2 ratio of about 75:25%. However, isoprenaline failed to increase heart rate in isolated, spontaneously beating right atria and failed to increase force of contraction in isolated, electrically driven right ventricular preparations of the β_1-adrenoceptor knockout but not the WT mice. Moreover, cardiac adenylyl cyclase was not at all stimulated in the β_1-adrenoceptor knockout mice, whereas in lung membrane preparations of the β_1-adrenoceptor knockout mice, β_2-adrenoceptor stimulation caused marked adenylyl cyclase activation. These results suggest that under normal physiological conditions, only β_1-adrenoceptors are functional in the mouse heart, which is in contrast to the human heart.

The β_1-adrenoceptor knockout mice show normal resting heart rate and blood pressure in vivo (Rohrer et al., 1996, 1998). However, this does not indicate that β_1-adrenoceptors do not play a role in control of resting heart rate in the mouse because 1) propranolol decreases resting heart rate in WT but not in knockout mice and 2) resting heart rate of the knockout mice was significantly lower than in WT mice in the presence of atropine (Rohrer et al., 1998). In vivo administration of isoprenaline caused normal hypotensive responses in the β_1-adrenoceptor knockout mice (via activation of vascular β_2-adrenoceptors) but a blunted tachycardic response and a complete lack in inotropic response (Rohrer et al., 1996). On the other hand, β_1-adrenoceptor knockout mice retain at least in part the baroreflex mechanism, and this might explain the small chronotropic effect of isoprenaline (Rohrer et al., 1998). The predominant, if not exclusive, role of β_1-adrenoceptors in the mouse heart is also supported by the findings that in β_2-adrenoceptor knockout mice, the heart rate response to isoprenaline is identical to that in WT mice (Chruscinski et al., 1999), whereas, as to be expected, the hypotensive response to isoprenaline is absent in these animals. Interestingly, in the β_1- and β_2-adrenoceptor double knockout mice, β_3-adrenoceptor stimulation seems to induce vasodilatation (Rohrer et al., 1999).

There are very few in vivo studies on the possible existence of a third β-adrenoceptor (β_3- or the putative β_4-adrenoceptor) in the human heart. Wheeldon et al. (1993) showed that the effects of isoprenaline infusion on heart rate and cardiac output (assessed with Doppler echocardiography to determine minute distance) were completely blocked by 20 mg of nadolol (a β_1- and β_2-adrenoceptor antagonist) in healthy young men, indicating that these effects were mediated by β_1- and/or β_2-adrenoceptors (see above). On the other hand, the isoprenaline-induced increase in systolic blood pressure and left ventricular stroke volume (assessed with Doppler echocardiography to determine stroke distance) was not attenuated by 20 mg of nadolol. In a subsequent study, these authors compared the effects of the β_2-adrenoceptor agonist salbutamol and the β_3-adrenoceptor agonist BRL 35135 (which is completely metabolized in vivo to the β_3-adrenoceptor agonist BRL 37344) on heart rate and minute distance in healthy young humans (Wheeldon et al., 1994). They found that both drugs increased heart rate and minute distance and that these changes were not affected by the β_1-adrenoceptor antagonist bisoprolol. However, although nadolol (20 mg) completely abolished the effects of salbutamol, a very small but significant increase in heart rate and minute distance evoked by BRL 35135 was resistant to this dose of nadolol (Wheeldon et al., 1994). Thus, according to these in vivo data, it cannot be completely ruled out that in the human heart, a functional non-β_1/non-β_2-adrenoceptor might exist. In this context, it is also worthwhile to note that it has been suggested that nonconventional β-adrenoceptor antagonists such as pindolol increase heart rate via a "third human cardiac β-adrenoceptor" (Kaumann, 1989). However, it should be noted that the partial agonistic activity of pindolol has been classified as a β_2-adrenoceptor agonist component in several systems. This has been shown in C_6 glioma cells containing β_1- and β_2-adrenoceptors where

long-term incubation of the cells with pindolol caused selective β_2-adrenoceptor down-regulation, whereas isoprenaline decreased the number of both β_1- and β_2-adrenoceptors (Neve et al., 1985). It has also been shown in rats where long-term treatment with pindolol for 7 days (by the use of Alzet minipumps) caused down-regulation of lung and cardiac β_2-adrenoceptors but not cardiac β_1-adrenoceptors (Hedberg et al., 1986). Finally, it has been shown in humans in vivo that long-term treatment with pindolol (and its derivatives mepindolol and bopindolol) caused down-regulation of β-adrenoceptors in circulating lymphocytes that express exclusively β_2-adrenoceptors (for references, see Brodde and Wang, 1988); moreover, a selective β_2-adrenoceptor down-regulation has also been described in right atria from patients chronically treated with pindolol (Michel et al., 1988). Thus, it is also possible that the effects of pindolol on heart rate are mediated by cardiac β_2-adrenoceptor stimulation.

D. Muscarinic Acetylcholine Receptors

Receptor cloning studies have demonstrated the existence of five different muscarinic receptor subtypes (m_1–m_5; Kubo et al., 1986a,b; Bonner et al., 1987, 1988; Peralta et al., 1987; see Fig. 5). Expressed in a variety of cells of mammalian/amphibian origin, these receptors exhibited functional properties that correspond to those previously defined by pharmacological criteria (for reviews, see Hulme et al., 1990; Caulfield, 1993). Thus, it has been recommended that the M_1, M_2, M_3, M_4, and M_5 nomenclature be used to describe both the pharmacological and the molecular subtypes (Caulfield and Birdsall, 1998). The chromosomal localization of the human M_1–M_5 receptor genes is 11q12-13, 7q35-36, 1q43-44, 11p12-11.2, and 15q26, respectively. In general, M_1, M_3, and M_5 receptors couple preferentially via $G_{q/11}$ to PLC with subsequent formation of inositol phosphates and diacylglycerol, whereas M_2 and M_4 receptors couple via a PTX-sensitive G protein (G_i/G_o) to inhibition of adenylyl cyclase (for reviews, see Felder, 1995; Wess, 1996). Additional signaling systems involved are effects on K^+ and Ca^{2+} channels and activation of phospholipase A_2, phospholipase D, and protein tyrosine kinases (Caulfield, 1993; Felder, 1995). The pharmacological characteristics of muscarinic receptor subtypes are shown in Table 4.

1. Muscarinic M_2 Receptors. There is general agreement that the predominant form of muscarinic receptors present in the heart of various mammalian species, including humans, is the M_2 receptor (Peralta et al., 1987; Hulme et al., 1990; Caulfield, 1993). Stimulation of these M_2 receptors mediates negative chronotropic and inotropic effects. In atria, stimulation of muscarinic receptors causes direct negative chronotropic and, in isolated tissues, inotropic effects. In ventricles, however, the negative inotropic effect can be demonstrated only when basal force of contraction has been enhanced in advance by cAMP-elevating agents, such as β-adrenoceptor agonists, forskolin, or phosphodiesterase inhibitors (i.e., the indirect, or "antiadrenergic", effect of muscarinic receptor agonists; for reviews, see Löffelholz and Pappano, 1985; Caulfield, 1993; Mery et al., 1997). However, in some species, such as in the ferret ventricular myocardium, acetylcholine can also exert direct inhibition of contractility (Ito et al., 1995).

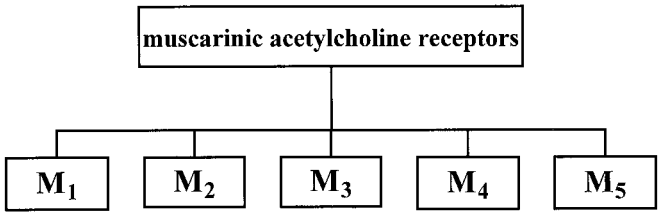

FIG. 5. Muscarinic receptor subtypes.

In the human heart, acetylcholine or carbachol decreased the basal force of contraction as well as the force of contraction stimulated by isoprenaline (Jakob et al., 1989; Deighton et al., 1990; Ungerer et al., 1990; Böhm et al., 1994; Du et al., 1994, 1995), noradrenaline (via β_1-adrenoceptors; Delhaye et al., 1984; Motomura et al., 1990a), procaterol (via β_2-adrenoceptors; Motomura et al., 1990a), and forskolin (Giessler et al., 1998) on isolated electrically driven human right atrial preparations. In ventricular preparations, however, acetylcholine or carbachol decreased only the force of contraction that had been previously activated by cAMP-elevating agents such isoprenaline or forskolin (Jakob et al., 1989; Böhm et al., 1990a, 1994; Deighton et al., 1990; Du et al., 1994, 1995; Koglin et al., 1994). Similarly, in vivo i.v. injection of carbachol reduced resting heart rate but not contractility, whereas carbachol reduced both parameters after stimulation of heart rate and contractility with isoprenaline (Von Scheidt et al., 1992b; Koglin et al., 1994). Furthermore, infusion of acetylcholine via the left main coronary artery did not affect basal force of contraction [assessed as peak (+)dP/dt] in humans but significantly attenuated increases in contractile force evoked by intracoronary infusion of the β-adrenoceptor agonist dobutamine (Landzberg et al., 1994; Newton et al., 1996). Moreover, the muscarinic receptor antagonist atropine does not affect resting force of contraction (Landzberg et al., 1994; Poller et al., 1997a,b; Schäfers et al., 1997), but it is well established that atropine increases resting heart rate.

One reason for these differences between atrial and ventricular myocardium might be the fact that parasympathetic innervation is much more dense in sinoatrial and atrioventricular nodes than in left ventricle of the human heart (Kent et al., 1974; Löffelholz and Pappano, 1985). However, the major reason may be a fundamental difference in the effector mechanisms activated by acetylcholine in atria and ventricles. In both atria and ventricles, activation of M_2 receptors, coupling to a PTX-sensitive G protein (G_i/G_o) leads to inhibition of adenylyl

TABLE 4
Pharmacological characterization of muscarinic receptor subtypes

	M_1	M_2	M_3	M_4	M_5
Potency order	Acetylcholine and carbachol do not discriminate subtypes				
Selective agonists					
Antagonists[a]					
Atropine	9.0–9.7	9.0–9.3	8.9–9.8	9.1–9.6	8.9–9.7
Pirenzepine	7.8–8.5	6.3–6.7	6.7–7.1	7.1–8.1	6.2–7.1
4-DAMP	8.6–9.2	7.8–8.4	8.9–9.3	8.4–9.4	8.9–9.0
Methoctramine	7.1–7.8	7.8–8.3	6.3–6.9	7.4–8.1	6.9–7.2
Himbacine	6.9–7.4	8.0–8.3	6.9–7.4	8.0–8.8	6.1–6.3
AF-DX 116	6.4–6.9	7.1–7.2	5.9–6.6	6.6–7.0	6.6
AF-DX 384	7.3–7.5	8.2–9.0	7.2–7.8	8.0–8.7	6.3
Tripitramine	8.4–8.8	9.4–9.6	7.1–7.4	7.8–8.2	7.3–7.5
pFHHSi D	7.2–7.5	6.0–6.9	7.8–7.9	7.5	7.0

4-DAMP, 4-diphenylacetoxy-*N*-methyl piperidine methiodide. Antagonist affinities are expressed as $-\log K_i$ or $-\log K_B$ values. Adapted from Alexander and Peters (1999), Birdsall et al. (1998), Caulfield (1993), Caulfield et al. (1998), and Eglen and Watson (1996).

[a] No subtype-selective agonists of high intrinsic efficacy are available; thus, muscarinic receptor subtypes are pharmacologically defined by a combination of several antagonists.

cyclase and hence inhibits increases in intracellular cAMP; this leads to a reduction in the L-type Ca^{2+} current (previously enhanced by cAMP) and appears to be the predominant mechanism of inhibiting force of contraction enhanced by cAMP-elevating agents (indirect inhibitory action; for recent reviews, see Hove-Madsen et al., 1996; Mery et al., 1997). In atrial myocytes (and in some Purkinje fibers), however, acetylcholine also opens a type of inwardly rectifying potassium channel ($I_{K.ACh}$) through direct effects of G protein α (for a review, see Kurachi, 1995; Wickman and Clapham, 1995; Clapham and Neer, 1997) or (preferentially?) $\beta\gamma$-subunits (for a recent review, see Yamada et al., 1998). This results in hyperpolarization, slowing of heart rate, shortening of the action potential duration, abbreviation of the L-type Ca^{2+} current, and reduction in the force of contraction (direct inhibitory action; Belardinelli and Isenberg, 1983). However, it may also be possible that G protein activation of acetylcholine-gated K^+ channels is achieved via additional mechanisms (see, for example, Kim et al., 1989). In human ventricular myocardium, on the other hand, stimulation of M_2 receptors has no direct negative inotropic effect, and it is still a matter of debate whether acetylcholine might activate the $I_{K.ACh}$ (see, for example, Koumi et al., 1997).

It has been known for a very long time that stimulation of muscarinic receptors in the heart can elevate cGMP levels (George et al., 1973; Goldberg et al., 1975; Inui et al., 1982), which might inhibit L-type Ca^{2+} currents via activation of cGMP-dependent protein kinase (Mery et al., 1991; for a recent review, see Lohmann et al., 1997) or via stimulation of cGMP-stimulated cAMP phosphodiesterase (Mery et al., 1993; for a recent review, see Mery et al., 1997). cGMP analogs can mimic the effects of acetylcholine in the heart, and it has been postulated that cAMP and cGMP levels are changed in an opposite direction in the heart (the "Ying Yang hypothesis"; Goldberg et al., 1975). However, a direct role for cGMP in the negative chronotropic and inotropic effects of muscarinic receptor stimulation has not been proved (for reviews, see Hartzell, 1988; Lohmann et al., 1991; Mery et al., 1997).

Recently, the existence of a constitutive endothelial NOS III has been demonstrated in cardiac myocytes (for a review, see Kelly et al., 1996). The major signaling pathway of NO is activation of a soluble guanylyl cyclase, thereby increasing intracellular levels of cGMP; this has led to the hypothesis that the acetylcholine-induced increase in cGMP might be due to stimulation of NOS activity (Kelly et al., 1996). In fact, in rabbit sinoatrial nodal (Han et al., 1994) and atrioventricular nodal (Han et al., 1996) cells, as well as in rat ventricular cardiomyocytes (Balligand et al., 1993, 1995), it has been shown that L-NMMA can antagonize acetylcholine-induced inhibition of L-type Ca^{2+} current. Moreover, Balligand et al. (1993) could show in neonatal rat ventricular cardiomyocytes that acetylcholine decreased spontaneous beating rate by 91%; this reduction was reversed by the NOS inhibitor L-NMMA and by methylene blue, an inhibitor of guanylyl cyclase, indicating that the effect of acetylcholine is, at least in part, mediated by NO, possibly via activation of guanylyl cyclase. 8-Bromo-cGMP could mimic the effects of acetylcholine. In addition, inhibition of NOS by L-NMMA and hemoglobin attenuated carbachol-induced reduction in contractility and L-type Ca^{2+} current in cardiomyocytes (Balligand et al., 1995). Moreover, NO was capable of inhibiting contraction of isolated, electrically driven guinea pig cardiomyocytes (Brady et al., 1993). In dogs, dobutamine-induced increases in the rate of beating and force of contraction were attenuated by vagal stimulation, and the effect of vagal stimulation could be in part attenuated by the NOS inhibitor L-NMMA (Hare et al., 1995a). Furthermore, in NOS III-knockout mice, Han et al. (1998) recently demonstrated that the isoprenaline-induced increase in L-type Ca^{2+} current and in force of contraction was not inhibited by acetylcholine, whereas acetylcholine potently inhibited isoprenaline effects in WT mice. Although these data appear to support the idea that NO plays an important role in the cardiac effects of acetylcholine, negative

results have also been reported. Thus, Vandecasteele et al. (1999), also using NOS III-knockout mice, failed to find any differences in muscarinic and β-adrenergic regulation of heart rate, force of contraction, and L-type Ca^{2+} currents in knockout versus WT mice. Furthermore, in frog ventricular cardiomyocytes, acetylcholine-induced inhibition of L-type Ca^{2+} current was completely unaffected by L-arginine analogs such as L-NMMA (Mery et al., 1993, 1996). The muscarinic inhibition of cAMP-stimulated Cl^- current in guinea pig ventricular cardiomyocytes was also unaffected by L-NMMA (Zakharov et al., 1996). Moreover, in isolated guinea pig ventricular cardiomyocytes, NOS was not involved in the cGMP-elevating effect of carbachol (Stein et al., 1993), and in isolated spontaneously beating rat right atria, L-NMMA did not affect the acetylcholine-induced negative chronotropic effect (Kennedy et al., 1994). In addition, one word of caution on the interpretation of results obtained with methylene blue and L-NMMA: both agents have been shown to be competitive muscarinic receptor antagonists (Buxton et al., 1993; Abi-Gerges et al., 1997; Pfaffendorf et al., 1997). Thus, it is still completely uncertain whether the NO-cGMP pathway plays an "obligatory role" for the muscarinic control of heart action (Han et al., 1994); the effects of the NO-cGMP system appear to be highly species dependent, and some of the above findings may require confirmation with NOS and/or guanylyl cyclase inhibitors that do not directly act on muscarinic receptors.

Only few data on the link between NO, acetylcholine, and negative inotropic effects in the human heart have been published. Intracoronary infusion of NO in humans reduced left ventricular pressure development and hastened left ventricular relaxation (Paulus et al., 1994). Hare et al. (1995b) reported in patients with varying degrees of left ventricular dysfunction that intracoronary infusion of L-NMMA potentiated the increase in left ventricular (+)dP/dt (as measure of inotropism) induced by simultaneous infusion of dobutamine. Similar results were obtained by Drexler et al. (1998), who demonstrated in left ventricular tissue from patients with end-stage heart failure that NOS II- activity was inversely correlated with inotropic responses to β-adrenoceptor stimulation. Moreover, L-NMMA enhanced the positive inotropic response to isoprenaline stimulation in failing hearts with high NOS II activity. Subsequently, Hare et al. (1998) could show that inhibition of cardiac NOS by L-NMMA increases positive inotropic responses to β-adrenergic stimulation only in patients with chronic heart failure—not in control subjects with normal left ventricular function. These results indicate that in chronic heart failure, NO might play a modulatory role in left ventricular function. However, it is still a matter of debate whether NOS II or NOS III activity might be increased in chronic heart failure (Stein et al., 1998).

On the other hand, in vitro studies on isolated, electrically driven human left ventricular myocardium failed to show any effect of L-NMMA or methylene blue on basal force of contraction, increases in force of contraction evoked by isoprenaline, or the antiadrenergic effect of carbachol (Kilter et al., 1995). Moreover, Vandecasteele et al. (1998) recently showed in isolated human right atrial myocytes that L-NMMA did not affect the acetylcholine-induced reduction in L-type Ca^{2+} current; moreover, in this preparation, NO donors did not reduce (as acetylcholine did) but rather enhanced L-type Ca^{2+} current (Kirstein et al., 1995; Vandecasteele et al., 1998). Divergent results have been reported for the effects of NO donors on the force of contraction in human isolated preparations. Nawrath et al. (1995) failed to show a significant effect of the NO-donor *S*-nitroso-*N*-acetyl-DL-penicillamine (SNAP) on isolated human right atrial preparations (it should be mentioned that numerous studies on this preparation have demonstrated a negative inotropic effect of acetylcholine or carbachol, see above), whereas 8-bromo-cGMP decreased the force of contraction. On the other hand, Flesch et al. (1997) demonstrated that the NO donor sodium nitroprusside and 8-bromo-cGMP decreased basal as well as isoprenaline-stimulated force of contraction in right atrial and left ventricular preparations of the failing and nonfailing human heart. The effects of sodium nitroprusside could be antagonized by methylene blue. Taken together, at the present, the majority of studies are not in favor of the idea that in the human heart, NO participates in the cardiac action of acetylcholine.

Finally, evidence has accumulated that acetylcholine can reduce isoprenaline-induced phosphorylation of cardiac regulatory proteins in the mammalian heart (e.g., phospholamban or troponin I; England, 1976; Lindemann and Watanabe, 1985; George et al., 1991; Neumann et al., 1994), suggesting that muscarinic receptor stimulation might activate protein phosphatases. In fact, Ahmad et al. (1989) have found that acetylcholine can increase the activity of protein phosphatases in guinea pig ventricular preparations. Moreover, Neumann et al. (1995) and Neumann and Scholz (1998) have shown that NaF (which can inhibit protein phosphatases) attenuated acetylcholine-induced "indirect" negative inotropic effects and significantly reduced activity of ventricular protein phosphatases. Similarly, the phosphatase inhibitor okadaic acid inhibited the decrease in phosphorylation state of cardiac regulatory proteins by acetylcholine (Gupta et al., 1994). In addition, Herzig et al. (1995) have recently shown that stimulation of protein phosphatases is involved in the inhibition of L-type Ca^{2+} channel by acetylcholine. The fact that the phosphatase inhibitor cantharidin increases force of contraction in failing and nonfailing human heart (Linck et al., 1996) indicates that protein phosphatases might also be involved in regulation of contractile force in human heart.

2. Is There Another Muscarinic, Non-M_2 Receptor in Human Heart?

As discussed, cardiac muscarinic receptors are predominantly of the M_2 subtype. Thus, radioligand bind-

ing and functional studies using several subtype-selective antagonists in isolated human atrial and ventricular preparations have detected only one binding site that had the typical characteristics of M_2 receptors (Giraldo et al., 1988; Deighton et al., 1990; Giessler et al., 1998). Moreover, in M_2 muscarinic receptor knockout mice, carbachol failed to reduce heart rate on isolated spontaneously beating right atria, whereas it produced a marked bradycardia in right atria from WT mice (Gomeza et al., 1999). However, in the heart of various species, it has been shown that muscarinic agonists at high concentrations (usually $>10^{-6}$ M, i.e., pharmacologic rather than physiologic concentrations) can induce a positive inotropic effect (Endoh et al., 1970; Brown and Brown, 1984; Korth and Kühlkamp, 1985, 1987; Webb and Pappano, 1995; Yang et al., 1996). This effect is PTX insensitive, often seen only after PTX treatment and appears to involve carbachol-induced induction of a tetrodotoxin-resistant inward Na^+ current (Korth and Kühlkamp, 1985; Matsumoto and Pappano, 1991). It has been speculated that this positive inotropic effect is mediated by an increase in inositol phosphate formation (for references, see Caulfield, 1993). Because M_2 receptor-mediated effects are generally regarded as PTX sensitive, it has been speculated that the increase in inositol phosphates might be due to M_2 receptor-mediated activation of G_i followed by release of $\beta\gamma$ complexes that can stimulate PLC, resulting in increased inositol phosphate formation (for references, see Wess, 1996). Alternatively, however, it is also possible that the increases in inositol phosphates and inotropic effects are mediated by a muscarinic receptor subtype different from M_2.

In favor of this idea are findings in guinea pig cardiac muscle (Ford et al., 1992) and neonatal rat ventricular cardiomyocytes (Sun et al., 1996) that muscarinic receptor agonist-induced inositol phosphate formation was inhibited by several muscarinic antagonists with an order of potency that did not fit with a characteristic of a M_2 receptor. Moreover, mRNA for M_1 receptors has been recently identified in rat and guinea pig ventricular cardiomyocytes; activation of these receptors with high concentrations of carbachol induced activation of PLC and increases in L-type Ca^{2+} currents (Gallo et al., 1993; Sharma et al., 1996, 1997; Colecraft et al., 1998). Finally, very recently, Shi et al. (1999) presented evidence that in atrial tissue of the dog heart, M_3 and M_4 receptors might have a functional role in the control of K^+ channels. On the other hand, Pappano and coworkers have quite convincingly demonstrated that in isolated ventricular myocytes of the guinea pig heart, only M_2 receptors are responsible for the stimulant effects of muscarinic agonists (Matsumoto and Pappano, 1991; Protas et al., 1998; Shen et al., 1999). Thus, it is still unclear whether a second muscarinic receptor subtype might exist in the mammalian heart.

The following lines of evidence are in favor of the idea that in addition to the M_2 receptor, a second subtype of muscarinic receptors might exist in the human heart. First, acetylcholine or carbachol not only decreases force of contraction but, in higher concentrations, also is able to increase contractile force in isolated human atria and ventricles (Du et al., 1994, 1995; Giessler et al., 1998) as has been shown in cardiac preparations of various animal species (see above). In atria, the decreases in contractile force are antagonized by the M_2 receptor antagonist AF-DX 116 much more potently than the M_1 receptor antagonist pirenzepine (Deighton et al., 1990; Du et al., 1995; Giessler et al., 1998), whereas the positive inotropic effect of acetylcholine or carbachol was not antagonized by AF-DX 116 but was significantly attenuated by pirenzepine (Du et al., 1995; Giessler et al., 1998). Furthermore, the M_1 receptor agonist McN-A-343 produced a positive inotropic effect in atria when contractility has previously been reduced by acetylcholine; this effect of McN-A-343 was antagonized by pirenzepine but not affected by AF-DX 116 (Du et al., 1995). These observations seem to indicate that in atria, the positive inotropic effect of muscarinic receptor agonists is mediated by a muscarinic receptor subtype different from the M_2. In ventricular myocardium, however, Du et al. (1995) found that the positive inotropic effect of acetylcholine is antagonized by AF-DX 116 but not by pirenzepine, indicating that this effect is mediated by M_2 receptor activation. Second, although the primary biochemical response to stimulation of muscarinic receptors in the human heart is inhibition of adenylyl cyclase, in higher concentrations, carbachol can enhance phosphoinositol turnover (Bristow, 1993). As discussed above, this might be due to M_2 receptor-mediated activation of G_i with subsequent release of $\beta\gamma$-subunit that stimulates PLC, but it might also be due to activation of a $G_{q/11}$-coupled muscarinic receptor (such as M_1 or M_3; Felder, 1995). Third, it has been known for a long time that low doses of atropine cause a negative chronotropic effect in humans (see, for example, Wellstein and Pitschner, 1988). This was initially attributed to central nervous system-stimulating effects of atropine, although this bradycardic effect has also been found with quaternary amines such as methylscopolamine bromide and atropine methylbromide (Kottmeier and Gravenstein, 1968). Similar effects have also been obtained with the rather M_1-selective antagonist pirenzepine: at low (M_1-selective) doses, heart rate is decreased, whereas at high (unselective) doses, heart rate is increased (Meyer and De Sommers, 1988; Pitschner and Wellstein, 1988; Poller et al., 1997a; Brodde et al., 1998a; Fig. 6). In contrast, the selective M_2 receptor antagonist AF-DX 116 only increased heart rate in healthy volunteers (Schulte et al., 1991). The mechanism underlying the paradoxical "cholinomimetic effect" of low doses of atropine and pirenzepine is not completely understood at the present. A central effect is not very likely because of the inability of pirenzepine to penetrate the blood-brain barrier (Carmine and Brogden, 1985). Another possibility

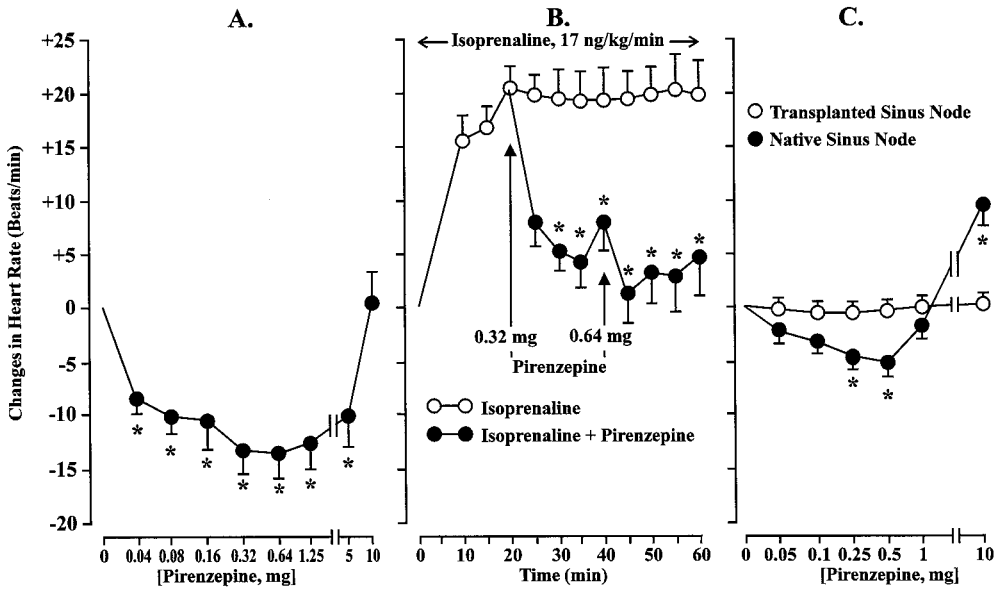

FIG. 6. Effects of pirenzepine in healthy volunteers on basal heart rate (A) and on heart rate increased by isoprenaline infusion (B) and in five heart transplant recipients on heart rate in the recipient's native (innervated) and transplanted (denervated) sinus node (C). A, pirenzepine was injected i.v. in six healthy male volunteers in eight incremental doses from 0.04- to 10-mg bolus each over 5 min, and heart rate was measured. Each dose step took 20 min. Values are means ± S.E.; *P <.05 versus baseline. B, pirenzepine (0.32- and 0.64-mg i.v. bolus) was injected 20 min after the start of continuous isoprenaline (17 ng/kg/min throughout the experiments) infusion in six healthy male volunteers, and heart rate was measured. Values are means ± S.E.; *P <.05 versus isoprenaline alone. C, pirenzepine was injected i.v. in five heart transplant recipients in six incremental doses from 0.05- to 10-mg bolus each over 5 min, and heart rate was measured in the recipient's native and transplanted sinus node. Each dose step took 20 min. Values are means ± S.E.; *P <.05 versus baseline. Data are from Poller et al. (1997a) and Brodde et al. (1998a) with some modifications.

could be M_1 receptor blockade of sympathetic ganglia (Meyer and De Sommers, 1988), thereby reducing release of noradrenaline and, hence, decreasing β-adrenoceptor-mediated heart rate increases. However, Pitschner and Wellstein (1988) showed that the negative chronotropic effect of pirenzepine can also be demonstrated when the volunteers were pretreated with high doses of propranolol. Thus, the most plausible explanation might be that atropine and pirenzepine inhibit presynaptic M_1 autoreceptors, thereby increasing the release of acetylcholine that causes the postsynaptic M_2 receptor bradycardic effects. In favor of this hypothesis are the findings in heart transplant recipients that the negative chronotropic effects of atropine (Epstein et al., 1990) and pirenzepine (Pitschner and Wellstein, 1988; Brodde et al., 1998a; Fig. 6) could be observed only with the recipient's native (i.e., innervated) sinus node but not with the transplanted (i.e., denervated) sinus node. Moreover, presynaptic muscarinic autoreceptors regulating the release of acetylcholine have been found in chicken, rat, rabbit, and guinea pig atria but not yet in human atria; they are species dependent of the M_1 (chicken: Jeck et al., 1988; Brehm et al., 1992) or M_2 (guinea pig: Jeck et al., 1988; rat: Bognar et al., 1990; rabbit: Habermeier-Muth et al., 1990) subtype. Taken together, there are some indications from in vitro and in vivo studies that another muscarinic receptor, one different from the M_2 subtype, may exist in the human heart, at least in right atrium; the final experimental proof, however, is still lacking.

III. Autonomic Responsiveness in the Aging Human Heart

Aging is associated with various changes in cardiovascular function. Because studies on alterations in α-adrenergic responses with aging are missing with regard to the human heart, this section mainly focuses on the cardiac β-adrenoceptor and aging, but alterations of muscarinic cardiac responsiveness with aging also are discussed.

A. β-Adrenoceptors

Numerous studies in animal models of aging, mainly in the rat, have shown that with increasing age, the cardiac response to β-adrenoceptor stimulation declines. On the other hand, studies in isolated rat ventricular myocytes have revealed that the positive inotropic effect induced by membrane-permeable cAMP analogs or increases in extracellular Ca^{2+} or by the Ca^{2+} agonist Bay K 8644 did not change with age (Sakai et al., 1992). These data indicate that the age-dependent decline in cardiac β-adrenoceptor responsiveness is obviously restricted to changes in the β-adrenoceptor/G protein/adenylyl cyclase system. Studies in aging myocardium of the rat did not show consistent changes in cardiac β-adrenoceptor number, but a general finding was that adenylyl cyclase activation by β-adrenoceptor agonists, GTP, NaF, and forskolin was significantly decreased in aged myocardium, suggesting changes in G proteins and/or catalytic unit of the adenylyl cyclase with aging

(Docherty, 1990; Lakatta, 1993a; Ferrara et al., 1997; Xiao et al., 1998).

Studies on age-dependent changes in the human β-adrenoceptor/G protein/adenylyl cyclase system have been performed mainly in circulating lymphocytes that contain a homogeneous population of β_2-adrenoceptors (Brodde et al., 1987). Most of these studies have not reported a decrease in lymphocyte β_2-adrenoceptor density with age but, in some agreement with the rat myocardium data (see above), a reduction occurred in lymphocyte cAMP response to various stimuli, including NaF and β-adrenoceptor stimulation (Feldman, 1986; Scarpace, 1986; O'Malley et al., 1988; Brodde, 1989), possibly due to a reduction in the activity of the catalytic unit of adenylyl cyclase (Abrass and Scarpace, 1982).

More recently, a few studies investigating in vitro age-dependent changes in β-adrenoceptors in the human heart have been published. White et al. (1994) studied ventricular β-adrenoceptor number and function in 26 nonfailing explanted human hearts from donors 1 to 71 years old. They found a significant decline in β-adrenoceptor number with age that was due predominantly to a loss in β_1-adrenoceptors (Fig. 7). Moreover, induction of the high-affinity state of the β-adrenoceptor (which is essential for coupling receptors to the effector system adenylyl cyclase) was significantly reduced with aging. In addition, ventricular adenylyl cyclase response to isoprenaline (activating β_1- and β_2-adrenoceptors) and to zinterol (activating β_2-adrenoceptors) decreased with aging. Similarly, guanylyl-5′-imidodiphosphate (GppC(NH)p, (acting at G_s and G_i proteins), NaF (acting predominantly at G_s protein) and forskolin (acting predominantly at the catalytic unit of the adenylyl cyclase but involving at least in part G_s) activation of adenylyl cyclase was reduced in the elderly (Fig. 7). On the other hand, Mn^{2+}-induced adenylyl cyclase activation (acting directly at the catalytic unit of the enzyme) was unchanged. These changes in β-adrenoceptor number and function were accompanied by unchanged PTX-catalyzed ADP-ribosylation but decreased cholera toxin-catalyzed ADP-ribosylation in ventricular membranes prepared from elderly versus younger hearts indicating no change in G_i protein but a decrease in G_s protein. However, measurement of left ventricular G_s protein by immunoblots did not reveal an age-dependent decrease in the amount of the α-subunit of G_s protein. Finally, on

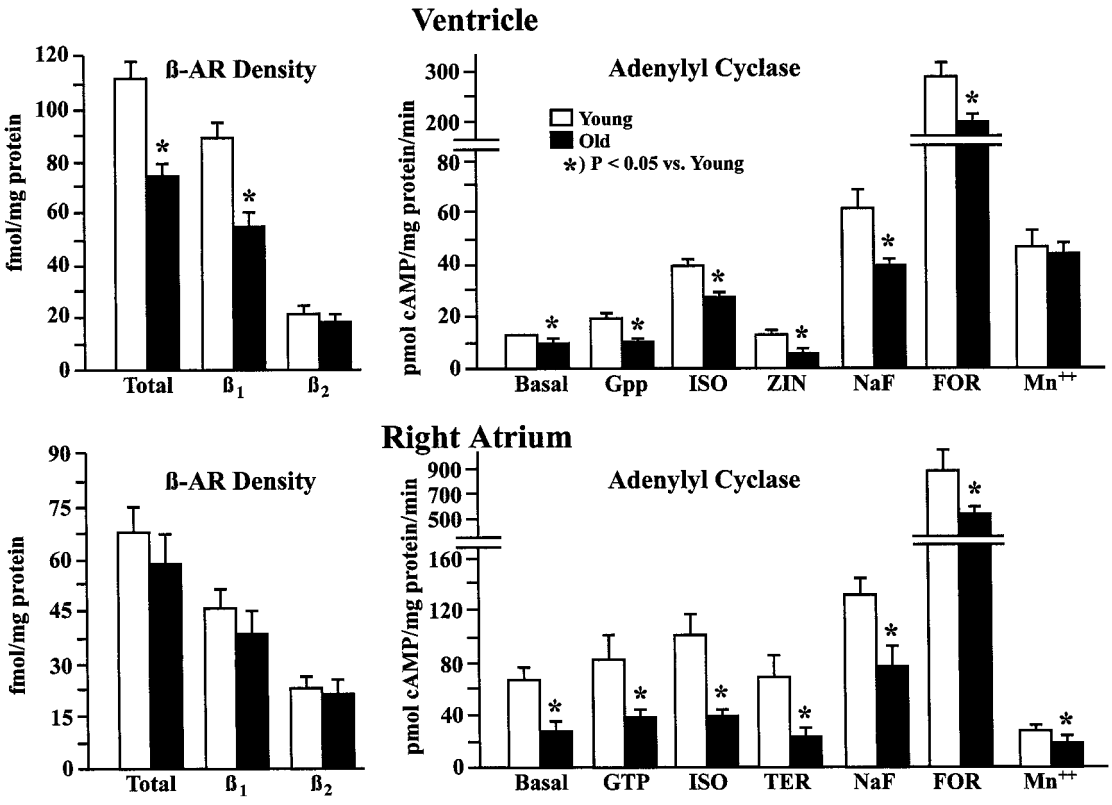

FIG. 7. Age dependence of the human myocardial β-adrenoceptor/adenylyl cyclase system. Top, β-adrenoceptor (AR) density [in fmol of (−)-[^{125}I]iodocyanopindolol (ICYP) specifically bound/mg protein] and adenylyl cyclase activity as net maximum stimulation (in pmol cAMP/mg protein/min) in ventricular membranes from nonfailing hearts of young (mean age, 20 ± 2.9 years; n = 13) and elderly (mean age, 50.4 ± 2.8 years; n = 13) organ donors. Values are means ± S.E. Gpp, 100 μM guanylyl-5′-imidodiphosphate − basal; ISO, 100 μM isoprenaline − GTP; ZIN, 100 μM zinterol − GTP; NaF, 10 mM NaF − basal; FOR, 100 μM forskolin − basal; Mn^{++}, 10 mM Mn^{2+} − basal. Data are recalculated from White et al. (1994). Bottom, β-adrenoceptor (AR) density [in fmol (−)-ICYP specifically bound/mg protein] and adenylyl cyclase activity as net maximum stimulation (in pmol cAMP/mg protein/min) in right atria obtained from young (mean age, 14.4 ± 2.1 years; n = 29) and elderly (mean age, 66.1 ± 1.5 years; n = 23) patients without apparent heart failure who were undergoing open heart surgery. Values are means ± S.E. GTP, 10 μM GTP − basal; ISO, 100 μM isoprenaline GTP; TER, 100 μM terbutaline − GTP; NaF, 10 mM NaF − basal; FOR, 100 μM forskolin − basal; Mn^{2+}, 10 mM Mn^{2+} − basal. Data are recalculated from Brodde et al. (1995b).

isolated electrically driven right ventricular trabeculae, the maximal positive inotropic response of isoprenaline was significantly reduced in those from elderly hearts, and the EC_{50} value for isoprenaline was increased by about 10-fold. On the other hand, the contractile response of these right ventricular trabeculae to high Ca^{2+} concentrations did not differ between young and elderly subjects. These results indicate that in human ventricular myocardium, the reduction in β-adrenergic responsiveness with age might be due to a decrease in β_1-adrenoceptor number; a reduction in G_s, which leads to an impaired cAMP formation, might contribute to this effect.

Harding et al. (1992) reported that the contractile response to isoprenaline was reduced in single ventricular myocytes from failing human hearts, and some portion of this reduction was related to the age of the patients. Subsequently, they could demonstrate in ventricular myocytes from 13 nonfailing human hearts (mainly patients with coronary artery disease without apparent heart failure aged 7 to 70 years) that the maximal contractile response to isoprenaline was significantly reduced in elderly patients (Davies et al., 1996a). In addition, there was a significant negative correlation between the age of the patients and the maximal contractile response to isoprenaline. Moreover, EC_{50} values for isoprenaline in elderly patients were about twice as high as in young subjects, although this difference did not reach statistical significance. On the other hand, the contractile response of these cardiomyocytes to high Ca^{2+} concentrations did not differ between young and elderly subjects, which is in agreement with the data from White et al. (1994) and the data obtained in aged rat myocardium (see above).

Brodde et al. (1995b) studied the β-adrenoceptor system in right atrial appendages from 52 patients of different ages (7 days to 83 years) without apparent heart failure who were undergoing open heart surgery. They found that neither β-adrenoceptor density nor subtype distribution changed with age; however, activation of right atrial adenylyl cyclase by isoprenaline, terbutaline (acting mainly at β_2-adrenoceptors), histamine (acting at H_2 receptors), serotonin (acting at serotonin 5-HT_4 receptors), GTP, NaF, forskolin, and Mn^{2+} declined with aging (Fig. 7). In addition, immunodetectable G_i increased with aging, whereas G_s remained unchanged. Finally, EC_{50} values for positive inotropic effects of isoprenaline on isolated electrically driven right atria obtained from children (mean age, 13 years) were about 10-fold lower than those in right atria obtained from elderly patients (age, >50 years). These results indicate that in human right atrium, the reduction in β-adrenergic responsiveness with age might involve a reduction in the activity of the catalytic unit of the adenylyl cyclase (similar to what has been observed in the human lymphocytes, see above), which leads to an impaired cAMP formation; the increase in G_i might enhance this effect.

Taken together, the available data clearly indicate that functional responses to β-adrenoceptor stimulation decrease with aging in the human heart. The underlying mechanisms, however, are not completely clear; according to the data available, in ventricular myocardium, a decrease in β_1-adrenoceptor number and G_s protein activity appears to be the major reason, whereas in right atria, the activity of the catalytic unit of the adenylyl cyclase decreases with aging, and this is accompanied by an increase in G_i. The reason for these differences between right atrial and ventricular myocardium is not known; however, it should be mentioned that ventricular tissue was obtained from organ donors excluded for heart transplantation who had been treated for 0.5 to 3 days with dopamine, whereas right atrial appendages were taken from patients undergoing open heart surgery without apparent heart failure who had not been treated with catecholamines at least 3 weeks before surgery. Nevertheless, independent of the underlying mechanism (age-dependent decrease in G_s or in the activity of the catalytic unit of adenylyl cyclase), the aging human heart shows reduced responses not only to β-adrenoceptor stimulation but also to stimulation of all receptors that mediate their effects via increases in the intracellular cAMP content. In this regard, the aging human heart is very similar to the failing human heart (see *Autonomic Responsiveness in the Failing Human Heart. B. β-Adrenoceptors*). However, it is not known whether the amount and activity of GRK2, which is increased in heart failure (Ungerer et al., 1993), might also be changed with age. A recent study in aged rat myocardium failed to detect any changes in abundance or activity of GRK2 or GRK5 (Xiao et al., 1998).

The cause of the age-dependent decline in cardiac β-adrenoceptor responsiveness is not completely understood. There is a progressive age-dependent loss of myocytes (Olivetti et al., 1991) that might explain the reduction in β-adrenoceptor responsiveness. Alternatively, it has repeatedly been shown that plasma noradrenaline levels are higher in elderly than in young people (Ziegler et al., 1976; for additional references, see Esler et al., 1990; Folkow and Svanborg, 1993; Lakatta 1993b), increasing by about 10 to 15% per decade (Esler et al., 1981). This elevation might reflect increasing sympathetic activity with aging as has been directly demonstrated from microneurographic recordings from sympathetic nerves to skeletal muscle (Mörlin et al., 1983; Ng et al., 1993). Thus, chronic elevation of plasma noradrenaline levels (and/or sympathetic activity) might induce β-adrenoceptor desensitization; this might also explain the reduction in β-adrenoceptor responsiveness with age.

In contrast to the in vitro data, numerous in vivo studies have been performed on age-dependent alterations in cardiac β-adrenoceptor responses. Two methods have mainly been used to study human cardiac β-adrenoceptor responsiveness in vivo: measurement of

exercise heart rate and heart rate responses to the β-adrenoceptor agonist isoprenaline. Exercise testing has been shown by many investigators to be a precise indicator for human cardiac β_1-adrenoceptor sensitivity (for reviews, see McDevitt, 1989; Brodde, 1991). Numerous exercise-testing studies on age-dependent changes in cardiac performance have been performed. The consistent result was that with increasing age, maximal heart rate is reduced with exercise stress (Julius et al., 1967; Port et al., 1980; Hossack and Bruce, 1982; Rodeheffer et al., 1984; Higginbotham et al., 1986; Schulman et al., 1992; Stratton et al., 1994; Fleg et al., 1995; for additional references, see Folkow and Svanborg, 1993; Lakatta, 1993b). Thus, these data are in agreement with the in vitro observations of a decreased β-adrenoceptor responsiveness in aging, although it should be mentioned that part of this reduction can be normalized by exercise training and increased activity (Bortz, 1982; Stratton et al., 1994; Cherubini et al., 1998).

The second generally used method to assess cardiac β-adrenoceptor responsiveness in humans is the heart rate response to isoprenaline. This was originally assessed with the use of rapid i.v. bolus injections of different doses of isoprenaline, thereby constructing dose-response curves (Cleaveland et al., 1972). Using this method, several groups have demonstrated that any given bolus dose of isoprenaline causes larger heart rate increases in young than in elderly subjects (Cleaveland et al., 1972; Vestal et al., 1979; Bertel et al., 1980; Van Brummelen et al., 1981; Kendall et al., 1982; Fitzgerald et al., 1984; Klein et al., 1986; Montamat and Davies, 1989), indicating a diminished responsiveness of cardiac β-adrenoceptors to isoprenaline. However, it has been shown with the use of continuous intra-arterial monitoring of blood pressure that bolus injection of isoprenaline not only increases heart rate but also decreases diastolic, systolic, and mean blood pressures; when the volunteers were pretreated with atropine to eliminate vagal effects, the isoprenaline-induced tachycardia was blunted, whereas blood pressure decreases were enhanced. These changes indicate reflex withdrawal of vagal tone, and this appears to largely contribute to the heart rate response to bolus injections of isoprenaline (Arnold and McDevitt, 1983). Accordingly, the heart rate response is markedly influenced by the fall in blood pressure and the efficiency of the arterial baroreflex. Because the baroreflex activity decreases with age (see below), an age-dependent blunting of baroreflex-mediated vagal withdrawal might mimic a decrease in β-adrenoceptor responsiveness with aging.

In subsequent studies, the effects of continuous infusion of different doses of isoprenaline were studied. This resulted not in a decrease but actually in an increase in vagal activity (i.e., in the presence of atropine, the dose-response curve for the increases in heart rate induced by isoprenaline is shifted to the left, opposite to what has been observed during bolus injections of isoprenaline; Arnold and McDevitt, 1984). Thus, when the effects of isoprenaline infusion on heart rate in young and elderly volunteers were investigated, similar chronotropic effects were observed in the two age groups, whereby the C_{25} of isoprenaline tended to be higher in the elderly (Klein et al., 1988; Stratton et al., 1992, 1994; White and Leenen, 1994; Brodde et al., 1998a; White et al., 1998). Similarly, no differences in the chronotropic effect of dobutamine were observed between young and elderly patients (Poldermans et al., 1995). However, when infusions were repeated after pretreatment of the subjects with the ganglionic blocker trimetaphan (White and Leenen, 1994; White et al., 1998), thus blocking compensatory reflexes, or with the anticholinergic drug atropine (Brodde et al., 1998a), thus blocking vagal tone, heart rate responses to isoprenaline were significantly larger in young than in elderly subjects (Fig. 8). These results indicate that 1) that the heart rate response to isoprenaline infusion is a mixture of increases induced by β-adrenergic stimulation and decreases induced by enhanced vagal tone and 2) the real effect of isoprenaline infusion on heart rate can be estimated only if vagal tone is blocked. Under the latter conditions, β-adrenergic heart rate responses are decreased in the elderly. Similar effects were recently observed for noradrenaline: in young and elderly volunteers, infusion of the amine caused nearly identical small decreases in heart rate; after pretreatment of the volunteers with atropine, heart rate markedly increased in young but not in the elderly volunteers (Poller et al., 1997b). On the other hand, White and Leenen (1997) recently reported that the heart rate response to adrenaline infusion was similar in young and elderly volunteers and was not affected by the ganglionic blocker trimetaphan (in contrast to the isoprenaline effects; Fig. 8). As discussed above, after exclusion of compensatory reflexes, age-dependent differences in cardiac β-adrenoceptor responsiveness can be demonstrated. The failure to do so with adrenaline might be due to the fact that adrenaline is activating heart rate in humans nearly exclusively via β_2-adrenoceptors (see *Presence and Function of Receptor Subtypes in Human Heart. β_1- and β_2-Adrenoceptors*; Fig. 4), whereas isoprenaline is acting at β_1- and β_2-adrenoceptors to about the same extent, and dobutamine and noradrenaline are acting predominantly at β_1-adrenoceptors (Daul et al., 1995; Schäfers et al., 1997). In addition, adrenaline is a substrate for neuronal uptake in human heart, whereas isoprenaline is not (Gilbert et al., 1989; Von Scheidt et al., 1992a), and evidence has accumulated that with aging, neuronal uptake of catecholamines declines (Esler et al., 1995; for additional references, see Folkow and Svanborg, 1993). Therefore, it also might be possible that the concentrations of adrenaline at the receptor site are higher in elderly compared with young volunteers due to a decreased neuronal uptake, thus compensating for the decreased β-adrenoceptor responsiveness. In fact, after pretreatment of the volunteers with tri-

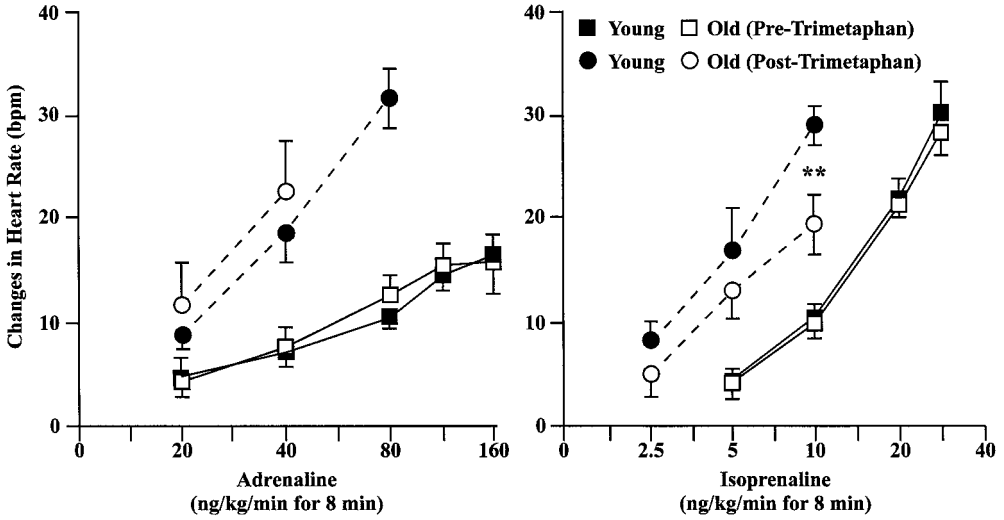

FIG. 8. Changes in heart rate in response to adrenaline infusion (left) and isoprenaline infusion (right) with or without trimetaphan in young (mean age, 30 ± 2 years; $n = 14$) and elderly (mean age, 60 ± 2 years; $n = 15$) healthy volunteers. Ordinate, increases in heart rate (in Δ bpm). Abscissa, dose of adrenaline (left) and isoprenaline (right) (in ng/kg/min). Values are means ± S.E.; $^{**}P < .02$, increases in heart rate induced by isoprenaline with concomitant trimetaphan were in young volunteers significantly larger than those in elderly volunteers. Isoprenaline data are from White and Leenen (1994); adrenaline data are from White and Leenen (1997).

metaphan plus desipramine, adrenaline-induced increases in heart rate were significantly larger in young than in elderly volunteers (F. H. H. Leenen, personal communication).

B. Muscarinic Receptors

Evidence has accumulated that aging is accompanied by a decrease in cardiac parasympathetic activity (Pfeifer et al., 1983; Fouad et al., 1984; O'Brien et al., 1986; Low et al., 1990). However, animal studies on cardiac M_2 receptor changes with age have resulted in divergent results. Thus, the density of M_2 receptors in rat heart has been reported to be unchanged (Narayanan and Derby, 1983; Elfellah et al., 1986; Narayanan and Tucker, 1986; Su and Narayanan, 1992; Böhm et al., 1993; Hardouin et al., 1997) or decreased (Chevalier et al., 1991). Moreover, carbachol inhibition of isoprenaline-activated adenylyl cyclase was significantly less in 24-month-old than in 6-month-old rats (Narayanan and Tucker, 1986). Finally, the decrease in heart rate induced by muscarinic agonists or vagal activation was found to be reduced (Rothbaum et al., 1974; Kelliher and Conahan, 1980), unchanged (Elfellah et al., 1986), or even increased (Ferrari et al., 1991; Su and Narayanan, 1992) in aged rats.

Only very few in vitro and in vivo studies have been performed to study muscarinic M_2 receptor changes in the human heart. Brodde et al. (1998a) recently investigated M_2 receptor density and function in right atria from 39 patients of different ages (5 days to 76 years). They found that M_2 receptor density declined with aging (Fig. 9), and there was a significant negative correlation between M_2 receptor density and the age of the patients. The decrease in M_2 receptor density was accompanied by a reduced ability of carbachol to inhibit forskolin-stimulated adenylyl cyclase; subsequently, this group could show that on isolated electrically driven human right atrial trabeculae prestimulated with forskolin, the negative inotropic effect of carbachol decreased with the age of the patients (Giessler et al., 1998).

The age-dependent decrease in human right atrial M_2 receptors is accompanied by an age-dependent bradycardic effect of M_2 receptor activation. Thus, Poller et al. (1997a) studied the effects of a wide range of doses of atropine and pirenzepine on basal heart rate in healthy volunteers aged 25 and 60 years. They found that the initial bradycardic effect of low doses of atropine and pirenzepine (Fig. 9) was significantly larger in the young than in the elderly volunteers. Similarly, Poller et al. (1997b) observed that the increase in resting heart rate induced by atropine was significantly lower in elderly versus young volunteers, thus confirming previously published data (Nalefski and Brown, 1950; Dauchot and Gravenstein, 1971). And finally, Brodde et al. (1998a) could show that the decrease in isoprenaline-stimulated heart rate with low doses of pirenzepine was significantly larger in young volunteers than it was in elderly volunteers. Taken together, these results are compatible with the view that with aging not only the number but also the in vivo function of M_2 receptors declines, at least in the right atrium. However, because the negative chronotropic effect of pirenzepine (and atropine) seems to involve prejunctional M_1 receptors (see *Is There Another Muscarinic, Non-M_2 Receptor in Human Heart?*), it is also possible that the decreased response to low doses of pirenzepine is due to a decreased M_1 receptor activity and/or due to a reduced amount of released acetylcholine (as has been observed in the rat heart; Meyer et al., 1985). The age-dependent changes in M_2 receptor number and/or functional responsiveness might also be in-

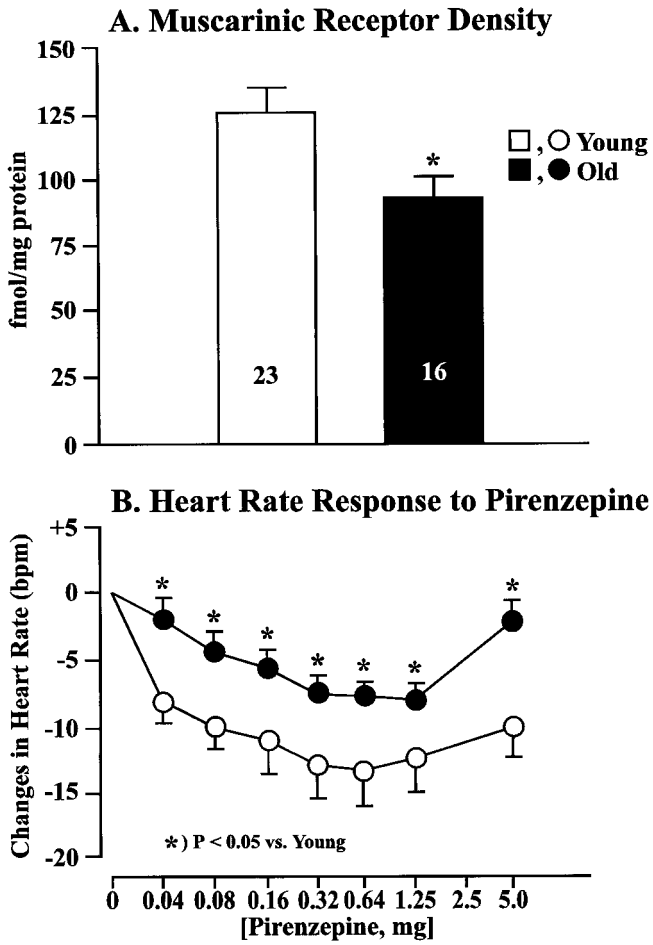

FIG. 9. Age dependence of human right atrial muscarinic receptors. A, muscarinic receptor density (in fmol of [^{3}H]*N*-methyl-scopolamine specifically bound/mg protein) in right atria obtained from young (mean age, 17.2 ± 2.1 years; n = 23) and elderly (mean age, 66.8 ± 1.8 years; n = 16) patients without apparent heart failure undergoing open heart surgery. Values are means ± S.E. Data are recalculated from Brodde et al. (1998a). B, effects of pirenzepine in six young (mean age, 26 ± 2 years) and six elderly (mean age, 60 ± 2 years) healthy volunteers on basal heart rate. Pirenzepine was injected i.v. in seven incremental doses from 0.04- to 5.0-mg bolus each over 5 min, and heart rate was measured. Each dose step took 20 min. Data are means ± S.E. modified from Poller et al. (1997a).

volved in the well known decrease in baroreflex sensitivity with age (i.e., a decreased bradycardia to pressor agents often observed in aged subjects; Gribbin et al., 1971; McDermott et al., 1974; Duke et al., 1976; Collins et al., 1980; Parati et al., 1995; for additional references, see Docherty, 1990; Folkow and Svanborg, 1993; Lakatta, 1993a,b; Persson, 1996).

Taken together, the above data clearly demonstrate a reduced responsiveness of both β-adrenoceptors and muscarinic receptors in the human heart with aging. However, the molecular correlates of such desensitization are still under discussion.

IV. Autonomic Responsiveness in the Failing Human Heart

An increased activity of the sympathetic nervous system is a well known feature in patients with chronic heart failure (Packer, 1992); thus, plasma noradrenaline levels are elevated in patients with heart failure (Cohn, 1995). This seems to result from increased cardiac noradrenaline spillover due to enhanced cardiac sympathetic drive (for a recent review, see Esler et al., 1997) and from decreased neuronal catecholamine uptake within the heart (Böhm et al., 1995; Eisenhofer et al., 1996). In addition, cardiac noradrenaline stores are depleted (Anderson et al., 1992). The increased cardiac sympathetic drive that appears to occur very early in heart failure (Rundqvist et al., 1997) results in long-term exposure of cardiac adrenoceptors to increased agonist concentrations. This can be expected to alter cardiac adrenoceptor responsiveness. Because adrenoceptors provide the main means for control of human cardiac contractility (Brodde et al., 1995a), such alterations are of obvious clinical relevance and therefore have been investigated in great detail, specifically for β-adrenoceptors. In addition, a chronically enhanced sympathetic drive to the heart may have toxic effects on cardiomyocytes (see *Autonomic Responsiveness in the Failing Human Heart. B. β-Adrenoceptors*).

A. α_1-Adrenoceptors

A reduced β-adrenoceptor density is well established in the failing human heart (Brodde, 1991), but a similar reduction does not appear to occur for α_1-adrenoceptors. Thus, only one study has reported a reduced α_1-adrenoceptor number in this disease state (Limas et al., 1989a), whereas three studies did not report significant changes (Böhm et al., 1988; Bristow et al., 1988; Hwang et al., 1996) and four studies even reported a doubling of α_1-adrenoceptor density (Vago et al., 1989; Steinfath et al., 1992b; Hwang et al., 1996; Yoshikawa et al., 1996). The reasons for this disagreement are not fully clear. However, the study reporting a reduced α_1-adrenoceptor number in congestive heart failure detected similar reductions in α_1-adrenoceptor number in the sarcolemmal membrane and the light vesicular fraction (Limas et al., 1989a), indicating that receptor sequestration into intracellular compartments did not explain the apparent reduction. Moreover, the underlying cause of congestive heart failure may be important. Thus, one study has detected an increase in α_1-adrenoceptor expression in ischemic but not in dilated cardiomyopathy relative to controls (Hwang et al., 1996). Taken together, these data indicate that if anything, human heart failure is associated with a slightly increased cardiac α_1-adrenoceptor number.

This apparent lack of α_1-adrenoceptor down-regulation in congestive heart failure has led to the hypothesis that in severe heart failure, α_1-adrenoceptors might be an inotropic back-up system in face of the diminishing β-adrenergic responsiveness. However, this hypothesis is not supported by the functional data: the positive inotropic effects of α_1-adrenoceptor stimulation in vitro have been reported to be unchanged (Böhm et al., 1988) or even reduced in congestive heart failure (Steinfath et

al., 1992b), even though receptor number was increased in the same hearts of the latter study. Similarly, intracoronary infusion of phenylephrine was reported to produce smaller inotropic effects in vivo in heart failure patients than in those with normal left ventricular function (Landzberg et al., 1991). At the biochemical level, it was shown that increased α_1-adrenoceptor number in failing hearts was not associated with similar enhancements of receptor function, because activation of the large G_h G protein remained unaltered due to a reduction in G_h in the membrane fraction (Hwang et al., 1996). Interestingly, the G protein classically associated with α_1-adrenoceptors (i.e., G_q) does not appear to be altered quantitatively in human congestive heart failure (Pönicke et al., 1998); in addition, α_1-adrenoceptor stimulation-induced inositol phosphate formation seems to be unchanged in chronic heart failure (Bristow, 1993). Taken together, these data indicate a defective receptor-effector coupling of cardiac α_1-adrenoceptors in human heart failure. They do not support the former hypothesis that α_1-adrenoceptors could constitute an inotropic back-up system that steps in when β-adrenoceptor-mediated inotropic effects are desensitized.

B. β-Adrenoceptors

Alterations in the β-adrenoceptor system in chronic heart failure have been the subject of several extensive reviews (Feldman and Bristow, 1990; Brodde, 1991; Bristow, 1993, 1997; Harding et al., 1994; Böhm, 1995; Brodde et al., 1995a; Ferrara et al., 1997) and are described here only briefly: In chronic heart failure, there is a substantial decrease in cardiac β_1-adrenoceptors (that occurs on the protein and mRNA level), an uncoupling of cardiac β_2-adrenoceptors (but often with no change in number or mRNA levels), no change in the amount and functional activity of cardiac G_s, an up-regulation of the activity and (in most but not all studies) amount of cardiac G_i, an up-regulation of mRNA levels and phosphorylation activity of cardiac GRK2, and no change in the activity of the catalytic unit of adenylyl cyclase and of PKA. In addition, autoantibodies directed against cardiac β_1-adrenoceptors have been detected in patients with chronic heart failure; in most, but not all, studies these antibodies appeared to activate the β_1-adrenoceptors (Limas et al., 1989b; Magnusson et al., 1994; Wallukat et al., 1995; Podlowski et al., 1998; Jahns et al., 1999). Nothing is known on the possible alterations of β_3-adrenoceptors (if present) or the putative β_4-adrenoceptors. The consequence of these pathological processes that are associated with alterations in Ca^{2+} handling in the failing human heart (for a recent review, see Davies et al., 1996b; Drexler et al., 1997; Hasenfuss, 1998) is the well known decreased cardiac β-adrenoceptor functional responsiveness that has been demonstrated in numerous in vitro (on isolated cardiac preparations) and in vivo studies. This decreased inotropic (and chronotropic) responsiveness of the failing human heart to β-adrenoceptor stimulation might also be responsible for the reduction in maximal exercise capacity (White et al., 1995). Moreover, a recent study in atrial and ventricular preparations of end-stage failing human hearts demonstrated that responses not only 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 presumably is due to increased activity of G_i that mitigates cAMP 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). Thus, as mentioned earlier (see *Autonomic Responsiveness in the Aging Human Heart. A. β-Adrenoceptors*), the failing human heart shows some similarities with the aging human heart: in both settings, responses to stimulation of all receptors that involve increases in intracellular levels of cAMP are diminished (also see Ferrara et al., 1997).

In addition to desensitization of the cardiac β-adrenoceptor system, the enhanced cardiac sympathetic drive in patients with chronic heart failure appears to exert toxic effects on the cardiomyocytes (for a recent review, see Colucci, 1997). It was known for a long time that high concentrations of catecholamines are toxic to the heart (for a review, see Rona, 1985). Mann et al. (1992) have directly shown in isolated ventricular cardiomyocytes of the rat in vitro that noradrenaline exerts toxic effects on the cardiomyocytes via a β-adrenoceptor-mediated pathway. Moreover, recent studies in the rat in vivo (Shizukuda et al., 1998) and in isolated adult rat ventricular cardiomyocytes in vitro (Communal et al., 1998) have shown that catecholamines can stimulate programmed cell death (apoptosis); this again is mediated by activation of cardiac β-adrenoceptors. In the failing human heart, myocyte necrosis and apoptosis have been demonstrated and have been considered to contribute significantly to progression of the disease (for recent reviews, see Anversa and Kajstura, 1998; Haunstetter and Izumo, 1998).

C. Muscarinic Receptors

In contrast to sympathetic tone, evidence has accumulated that in chronic heart failure, vagal activity is decreased (Eckberg et al., 1971; Porter et al., 1990; La Rovere et al., 1994). Nevertheless, the majority of studies did not find considerable changes in the number and function of muscarinic receptors in the failing human heart (as assessed by inhibition of adenylyl cyclase activity and negative inotropic effects in isolated cardiac preparations; Böhm et al., 1990a,b; Brodde et al., 1992; Fu et al., 1992; Bristow, 1993; Pönicke et al., 1998; Giessler et al., 1999). Similarly, in vivo intracoronary acetylcholine inhibited intracoronary dobutamine-induced positive inotropic effects [assessed as (+)dP/dt] in patients with dilated cardiomyopathy to a very similar extent as in subjects with normal ventricular function

(Newton et al., 1996; Hare et al., 1998). Only a recent in vivo positron emission tomography study using [^{11}C]methylquinuclidinyl benzilate as ligand found cardiac muscarinic receptors to be slightly but significantly higher in patients with congestive heart failure than in healthy controls (Le Guludec et al., 1997). The lack of significant changes of M_2 receptors in chronic heart failure is somewhat surprising because human cardiac M_2 receptors couple to G_i (see *Presence and Function of Receptor Subtypes in Human Heart. D1. Muscarinic M_2 Receptors*), 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 a discussion, see Brodde et al., 1995a). In this context it is interesting to note that Eschenhagen et al. (1996) recently showed in rats that chronic treatment with carbachol decreased not only cardiac 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, long-term treatment of the rats with isoprenaline, which causes increases in ventricular G_i, rather decreased the incidence in isoprenaline- and forskolin-induced arrhythmias. Subsequently, this group could show that in rats, inactivation of G_i protein by PTX treatment markedly increased the arrhythmogenic effects of isoprenaline (Grimm et al., 1998). 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.

D. Possible Mechanisms of Beneficial Effects of β-Blockers in Patients with Chronic Heart Failure

Because the chronically increased cardiac sympathetic drive in the failing human heart causes deleterious adverse biological effects on the cardiac myocyte via stimulation of the β-adrenergic pathway (see above), it is plausible that treatment of these patients with β-adrenoceptor antagonists might prevent these effects. In fact, during the past 20 years, several studies have convincingly demonstrated that long-term treatment of patients with chronic heart failure with β-adrenoceptor antagonists has beneficial effects (Bristow, 1997; Doughty and Sharpe, 1997; Krum, 1997; Lechat et al., 1998). One possible mechanism of the beneficial effects of β-adrenoceptor antagonists could be that they up-regulate the (previously down-regulated, see above) cardiac β-adrenoceptors. Because the human heart contains only a few spare β-adrenoceptors (for references, see Brodde et al., 1995a), such an up-regulation would be helpful in restoring maximal contractile responses to β-adrenoceptor stimulation. In fact, some β-adrenoceptor antagonists, such as metoprolol, have been shown to up-regulate β-adrenoceptors in the hearts of patients with chronic heart failure (Heilbrunn et al., 1989; Waagstein et al., 1989; Gilbert et al., 1996; Sigmund et al., 1996). Interestingly, several studies have shown that long-term treatment of patients with coronary artery disease with β_1-selective adrenoceptor antagonists such as metoprolol, atenolol, or bisoprolol sensitizes cardiac β_2-adrenoceptor function in vitro (Hall et al., 1990; Motomura et al., 1990a) and in vivo (Hall et al., 1991). Long-term β_1-adrenoceptor antagonist treatment also appears to sensitize cardiac H_2 histamine (Sanders et al., 1996) and serotonin 5-HT_4 receptors (Sanders et al., 1995). Whether this also occurs in patients with chronic heart failure and whether this may contribute to the beneficial effects of β_1-adrenoceptor antagonists in patients with chronic heart failure remain matters of debate. It also is not known which mechanisms underlie the "cross-talk" between human cardiac β_1- and β_2-adrenoceptors (but see below).

On the other hand, β-adrenoceptor up-regulation cannot be the sole mechanism for the beneficial effects of β-adrenoceptor antagonists in chronic heart failure (Fig. 10), because carvedilol (a nonselective β-adrenoceptor antagonist with considerable α-adrenoceptor antagonis-

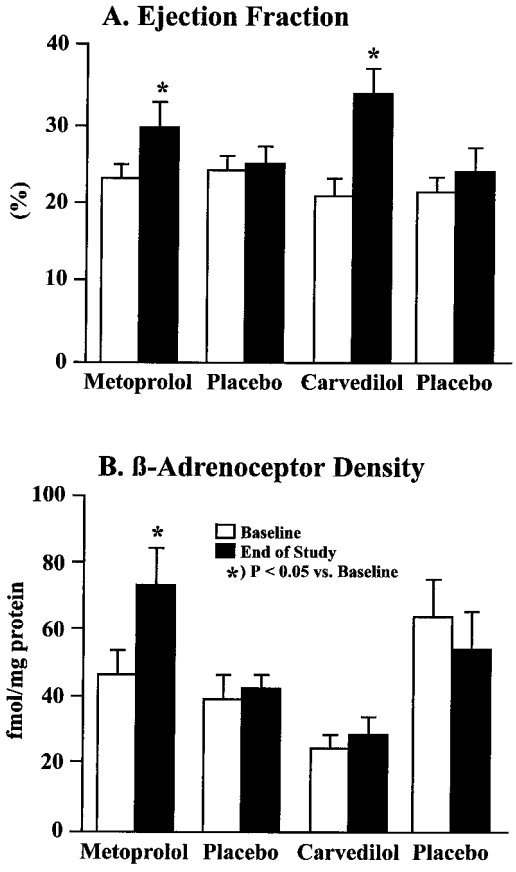

FIG 10. Left ventricular ejection fraction (A) and right ventricular endomyocardial β-adrenoceptor density (B) in patients with mild-to-moderate heart failure treated with metoprolol (125–150 mg/day) or carvedilol (50–100 mg/day) or their matching placebos. Ordinate: A, left ventricular ejection fraction (in %); B, right ventricular endomyocardial β-adrenoceptor density [in fmol of (−)-[^{125}I]iodocyanopindolol specifically bound/mg protein]. Note that both treatment regimens significantly increased left ventricular ejection fraction, whereas only metoprolol significantly increased β-adrenoceptor density. Values are means ± S.E. Data are recalculated from Gilbert et al. (1996).

tic and vasodilator properties, which has been shown to be very effective in patients with chronic heart failure; see Bristow, 1998), does not up-regulate cardiac β-adrenoceptors (Gilbert et al., 1996).

As mentioned above, activating autoantibodies against cardiac β_1-adrenoceptors have been detected in patients with chronic heart failure; these autoantibodies can down-regulate cardiac β_1-adrenoceptors (Podlowski et al., 1998), and this might contribute to the progression of the disease. β_1-Adrenoceptor antagonists can prevent the agonistic effects of these autoantibodies (Jahns et al., 1999), and this could (at least in some of the patients) contribute to the beneficial effects of β-adrenoceptor antagonist treatment in patients with chronic heart failure.

Another possible mechanism for the beneficial effects of β-adrenoceptor antagonists could be that they normalize the activity (and amount) of cardiac G_i. As discussed above, G_i is increased in chronic heart failure, and this might contribute to the fact that positive inotropic effects of all drugs acting via increases in intracellular cAMP are reduced (see above). Evidence has accumulated that the increase in cardiac G_i is the consequence of the prolonged exposure to high levels of catecholamines (for references, see Harding et al., 1994; Brodde et al., 1995a). One study in patients with congestive heart failure has indeed shown that long-term treatment with metoprolol led to a significant reduction in the amount of $G_{i\alpha}$ (assessed by PTX-catalyzed ADP-ribosylation; Sigmund et al., 1996). Whether this may contribute to the clinical improvement seen in patients with chronic heart failure during long-term β-adrenoceptor antagonist treatment is not clear at the present. However, a recent study from Böhm et al. (1997) has shown that in patients with chronic heart failure, 6-month treatment with metoprolol significantly improved the inotropic response to the cAMP-dependent phosphodiesterase inhibitor milrinone. Because milrinone increases cardiac performance independent of β-adrenoceptor stimulation, it might well be that the metoprolol-induced decrease in G_i (see above) is involved in the improved milrinone response.

In normal human subjects, increases in heart rate lead to enhanced left ventricular myocardial contractility (positive force-frequency relationship: Bowditch-Treppe phenomenon; Bowditch, 1871), and this positive force-frequency effect is enhanced by β-adrenoceptor stimulation (Ross et al., 1995; Bhargava et al., 1998). Patients with chronic heart failure show marked reduction in the positive force-frequency relationship (for references, see Just, 1996) and no augmentation by β-adrenoceptor stimulation (Bhargava et al., 1998). β-Adrenoceptor antagonists decrease heart rate; this might shift the force-frequency relationship toward lower rates of beating and, by this, may improve contractility in patients with chronic heart failure.

As mentioned, one typical alteration in the failing human heart is an increase in mRNA and activity of GRK2. Recent studies, mainly in transgenic mice (see *Lessons from Transgenic Animals*), have shown that GRK2 appears to play an important role in the regulation of myocardial contractile function. An increase in the activity of GRK2 (as in chronic heart failure patients) is obviously associated with a decrease in contractile force, and this can be restored by inhibition of GRK2 (see *Lessons from Transgenic Animals*). In vivo studies in pigs have shown that treatment with the β_1-adrenoceptor antagonist bisoprolol causes down-regulation of GRK2 (Ping et al., 1995). A recent study in mice has shown that long-term infusion of isoprenaline decreases cardiac β-adrenoceptor signaling and increases GRK2 activity, thus presenting evidence that, in fact, enhanced β-adrenoceptor stimulation can increase GRK2 activity. On the other hand, treatment of the mice with the β-adrenoceptor antagonists atenolol and carvedilol decreased GRK2 activity (Iaccarino et al., 1998b). Thus, it might well be possible that part of the beneficial effects of β-adrenoceptor antagonists in treatment of chronic heart failure is due to a reduction in the (previously enhanced) GRK2 activity; however, the experimental proof of this hypothesis is still lacking.

Finally, it should be mentioned that there are differences in the antiadrenergic effects of different β-adrenoceptor antagonists. Thus, two recent studies have shown that in patients with chronic heart failure, the nonselective β-adrenoceptor antagonists propranolol (Newton and Parker, 1996) and carvedilol (Gilbert et al., 1996) decreased cardiac noradrenaline spillover and coronary sinus noradrenaline levels, respectively, whereas the β_1-adrenoceptor selective antagonist metoprolol rather tended to increase both parameters. Obviously, blockade of presynaptic cardiac β_2-adrenoceptors (which have been shown to exist in the human heart and to mediate increased noradrenaline release; see *Presence and Function of Receptor Subtypes in Human Heart. C1. β_1- and β_2-Adrenoceptors*) might contribute to the beneficial effects of nonselective β-blockade with carvedilol in the treatment of chronic heart failure.

Taken together, marked alterations in the β-adrenoceptor/G protein/adenylyl cyclase/GRK2 cascade occur in human congestive heart failure, whereas alterations in α_1-adrenoceptors and muscarinic receptors appear to have a much smaller, if any, pathophysiological role in this disease. Although a beneficial effect of chronically administered β-adrenoceptor antagonists in patients with congestive heart failure is now well documented, the molecular mechanisms underlying these effects remain to be clarified.

E. Lessons from Transgenic Animals

Recombinant DNA technology has allowed not only the cloning of receptor genes for autonomic neurotransmitters but also the study of their function in defined biological environments, such as on transfection into cultured cells at desired expression levels. Furthermore, it has become possible to generate animals (largely mice)

that either lack a specific receptor or are transgenic and express a recombinant receptor in one or more targeted tissues (Wei, 1997). Obviously, such techniques can yield important insights into the physiological role of specific receptor subtypes. Before we discuss results from work in transgenic animals, some general limitations of these approaches should be discussed. Thus, the transgenic expression of a receptor or another protein in the heart can tell us what this protein can potentially do to cardiac physiology. However, physiological expression of the endogenous receptor usually occurs at much lower densities and thus may not always have the same functional consequences, particularly at the quantitative level. Although this limitation does not apply to knockout animals, both transgenic and knockout animals usually carry or lack the protein of interest from an early stage of life. Therefore, some of the alterations seen in such animals may represent complex adaptational responses rather than direct consequences of the presence or absence of the protein of interest.

As discussed above, alterations in the human cardiac β-adrenoceptor/G protein(s)/adenylyl cyclase system appear to play an important role in development and/or progression of chronic heart failure. Transgenic mouse models overexpressing components of the β-adrenoceptor/G protein(s)/adenylyl cyclase system have been created to study the role of the β-adrenoceptor system for cardiac function in vivo. Moreover, these studies could help us to understand whether overexpression of one of the components of the β-adrenoceptor system might be involved in the development of cardiac failure and/or whether the β-adrenoceptor gene might be a possible target for the application of gene therapy in heart failure.

The first study describing an altered myocardial β-adrenoceptor expression was performed by Bertin et al. (1993). These authors used the human ANF promotor to overexpress by 5- to 10-fold the human β_1-adrenoceptor in the atria of mice. This resulted in only minimally altered β-adrenergic signaling (Bertin et al., 1993). However, it was subsequently found that basal atrial contractility was increased and there was no isoprenaline-induced positive inotropic effect, whereas basal and isoprenaline-stimulated adenylyl cyclase activity was actually lower than that in the WT counterparts (Mansier et al., 1996). Subsequently, using the human β_1-adrenoceptor linked to the murine α-myosin heavy chain promotor, Port et al. (1998) and Engelhardt et al. (1999) succeeded in overexpressing β_1-adrenoceptors in all chambers of the mouse heart (by about 20- to 50-fold and 5- to 15-fold, respectively). These transgenic mice had increased cardiac contractility in young age but also developed marked hypertrophy (Engelhardt et al., 1999). In old mice, however, β-adrenoceptor function was markedly depressed compared with the WT counterparts; moreover, the transgenic mice showed marked ventricular dilation and reduced ejection fraction (Port et al., 1998; Engelhardt et al., 1999). Thus, it appears that cardiac overexpression of β_1-adrenoceptors initially leads to an improvement in cardiac function that is, however, followed by a progressive decrease in cardiac function, leading to heart failure.

Using the human β_2-adrenoceptor linked to the murine α-myosin heavy chain promoter, Milano et al. (1994a) produced transgenic mice overexpressing the β_2-adrenoceptor by >100 fold in all chambers of the mouse heart. Despite the fact that in WT mice heart rate and contractility are regulated predominantly (if not exclusively) via β_1-adrenoceptors (see *Is There a Third (or Fourth) β-Adrenoceptor Subtype Present in Human Heart?*), these animals markedly overexpressing β_2-adrenoceptors exhibited significantly higher indices of cardiac function (Bittner et al., 1997) and demonstrated maximal β_2-adrenoceptor signaling. Thus, in the absence of an agonist heart rate, (+)dP/dt and adenylyl cyclase activity were elevated to levels observed in WT animals after maximal stimulation with isoprenaline. Accordingly, in the transgenic animals, there was only very little additional β_2-adrenoceptor stimulation by isoprenaline. This might, at least in part, be due to the fact that in murine cardiomyocytes β_2-adrenoceptors couple to G_s and G_i (see *Presence and Function of Receptor Subtypes in Human Heart. C1. β_1- and β_2-Adrenoceptors*); in fact, PTX treatment could rescue contractile responses to β_2-adrenoceptor stimulation in ventricular myocytes of these transgenic mice (Xiao et al., 1999). On the other hand, these mice retained the heart rate response to nerve stimulation, and a small increase in (+)dP/dt was also detected during nerve stimulation (Du et al., 1996). Interestingly, although atenolol inhibited the effects of nerve stimulation on heart rate and (+)dP/dt in WT mice, the β_2-adrenoceptor antagonist ICI 118,551 did so in transgenic mice (Du et al., 1996). In addition, these mice exhibited markedly enhanced myocardial relaxation that could not be increased by isoprenaline and was accompanied by reduced myocardial phospholamban levels (Rockman et al., 1996b), indicating that long-term β-adrenergic stimulation can lead to decreased phospholamban protein levels. In this context, it is interesting to note that in a phospholamban knockout mouse, Ca^{2+} uptake into the sarcoplasmic reticulum was increased associated with increased myocardial contraction and relaxation that lacks isoprenaline responsiveness (Luo et al., 1994; Hoit et al., 1995). In contrast to the β_1-adrenoceptor-overexpressing mouse (see above), the cardiac β_2-adrenoceptor-overexpressing mouse appears not to develop heart failure, and even in old age, only minimal fibrosis was observed (Milano et al., 1994a; Peppel et al., 1997).

Subsequently, the β_2-adrenoceptor-overexpressing animals have been found to be a very suitable model to study the hypothesis of "inverse agonism" (see Milligan et al., 1995; Milligan and Bond, 1997). Constitutively active β-adrenoceptors are able to couple to G proteins

and evoke responses even in the absence of agonists. Inverse agonists, in contrast to neutral antagonists, inhibit both agonist-induced receptor activation (as neutral antagonists do) and constitutively active receptors. Inverse agonism is unmasked in systems with overexpression of β-adrenoceptors resulting in an increase in the number of constitutively active receptors. Using these transgenic mice, it was shown that atrial tension was maximally stimulated in vitro and isoprenaline did not cause further increases in contractile force; the β_2-adrenoceptor antagonist ICI 118,551 reduced basal atrial tension and restored isoprenaline-induced increases in contractile force to control levels in the transgenic but not in WT mice (Bond et al., 1995). It should be mentioned, however, that inverse agonism of β-adrenoceptor antagonists has also been demonstrated in native tissues (Mewes et al., 1993; Götze and Jakobs, 1994).

Recently, mice overexpressing GRK2 or a GRK2 inhibitor (the carboxyl terminus of GRK2 that competes for $G_{\beta\gamma}$ binding to the kinase, a process required for GRK2 activation; for review, see Zhang et al., 1997) were created (Koch et al., 1995). In animals overexpressing GRK2 by 3- to 5-fold, basal as well as isoprenaline-activated adenylyl cyclase activity was decreased. Moreover, the positive inotropic and chronotropic effects of isoprenaline were blunted in these transgenic animals. Similar effects on β-adrenergic receptor function also were observed in mice with cardiac-specific overexpression of GRK5 (Rockman et al., 1996a), whereas overexpression of GRK3 in the mouse heart did not affect β-adrenoceptor function (Iaccarino et al., 1998a). On the other hand, animals expressing the GRK2 inhibitor had increased basal left ventricular contractility in vivo and preserved responsiveness to isoprenaline but showed no signs of cardiac failure such as myocardial fibrosis or ventricular dilation. These data suggest that GRK2 might play an important role in regulation of cardiac β-adrenoceptor function. Subsequent studies appear to confirm this hypothesis. Rockman et al. (1998b) studied the effects of alterations in the level of GRK2 in two types of genetically altered mice: the first group was heterozygous for GRK2 knockout (the homozygous form of GRK2 knockout mice is lethal; Jaber et al., 1996), and the second group was heterozygous for GRK2 knockout plus transgenic for cardiac-specific overexpression of the GRK2 inhibitor. In the GRK2 knockout/GRK2 inhibitor animals, basal contractile force, as well as isoprenaline-induced increases in contractile force, was larger than that in heterozygous GRK2 knockout mice, which showed larger responses than WT mice. These results indicate that the levels of GRK2 play an important role in regulation of contractile force. Furthermore, in transgenic mice overexpressing GRK2, myocardial recovery after global ischemia and reperfusion was significantly impaired compared with WT mice (Chen et al., 1998). Moreover, in a mouse model of pressure overload with pronounced cardiac hypertrophy, cardiac β-adrenoceptors were markedly desensitized (as assessed by the contractile responses to dobutamine); this was accompanied by an about 3-fold increase in the activity of GRK2. Overexpression of the GRK2 inhibitor did not attenuate the development of cardiac hypertrophy but did prevent β-adrenoceptor desensitization (Choi et al., 1997). Finally, in a genetic model of murine-dilated cardiomyopathy (produced by gene-targeted disruption of the muscle LIM protein; Arber et al., 1997), which exhibits defective β-adrenoceptor signaling including increased GRK2 expression, overexpression of the GRK2 inhibitor prevented the development of cardiomyopathy, whereas overexpression of the β_2-adrenoceptor enhanced the heart failure symptoms (Rockman et al., 1998a). As mentioned, GRK2 is increased in human heart failure (Ungerer et al., 1993); this may be due to long-term stimulation of cardiac β_1-adrenoceptors [but not α_1-adrenoceptors (Iaccarino et al., 1999) and possibly β_2-adrenoceptors (Engelhardt et al., 1997)] by the enhanced sympathetic activity. Inhibition of GRK2 might be, therefore, a new and promising therapeutical strategy. In this context, it should be mentioned again that β-adrenoceptor antagonists appear to decrease GRK2 activity (see *Possible Mechanisms of Beneficial Effects of β-Blockers in Patients with Chronic Heart Failure*).

Overexpression of the α-subunit of G_s in the myocardium resulted in mice that exhibited cardiac supersensitivity to catecholamines (but not in the absence of catecholamines) due to increased $G_{s\alpha}$ protein, increased coupling to adenylyl cyclase, and increased number of β-adrenoceptors in the "high-affinity state" (Gaudin et al., 1995). Over time, these animals develop a cardiomyopathy (Iwase et al., 1996, 1997) characterized by cardiac fibrosis, left ventricular dilation, reduction in left ventricular mechanical function, and increased myocyte apoptosis (Geng et al., 1999). Moreover, old (16-month-old) mice with $G_{s\alpha}$ overexpression did not show the classic homologous desensitization after long-term stimulation with agonists: although β-adrenoceptor number was reduced and GRK2 levels were still increased in these old animals, the percentage of β-adrenoceptors in the high-affinity state was increased, and isoprenaline- and guanylyl-5′-imidodiphosphate-stimulated adenylyl cyclase was enhanced compared with age-matched controls (Vatner et al., 1998). Moreover, long-term isoprenaline treatment failed to elicit β-adrenoceptor desensitization in these animals. This lack of β-adrenoceptor desensitization and a depressed heart rate variability and arterial baroreflex (Uechi et al., 1998) might therefore contribute to the development of cardiac failure.

Taken together, these results indicate that elevated β-adrenoceptor signaling, due to either overexpressed β_1-adrenoceptors or overexpressed $G_{s\alpha}$ (combined with an impaired β-adrenoceptor desensitization in the latter case), initially increases cardiac function but in the long term is harmful to cardiac function and might be involved in the development and/or progression of heart

failure. The failure of overexpression of β_2-adrenoceptors to induce heart failure in the mouse is possibly due to the fact that this receptor subtype has, under normal conditions, no functional importance in the mouse heart (in contrast to the human heart; see *Presence and Function of Receptor Subtypes in Human Heart. C1. β_1- and β_2-Adrenoceptors*). In addition, overexpressed β_2-adrenoceptors in the mouse heart couple to G_i, which obviously inhibits β_2-adrenergic signaling (Xiao et al., 1999). However, it should be mentioned that at least in the genetic model of murine-dilated cardiomyopathy (the MLP knockout mouse; Arber et al., 1997), cardiac overexpression of the β_2-adrenoceptor worsens heart failure symptoms (Rockman et al., 1998a). Accordingly, it appears to be doubtful whether β-adrenoceptor gene transfer into the heart [which has been shown in nonfailing and failing rabbit cardiomyocytes (Akhter et al., 1997b; Drazner et al., 1997) and in pressure-overloaded rat heart (Kawahira et al., 1998) to increase cardiac β-adrenoceptor responsiveness] might be a promising approach for a long-term improvement of cardiac function in chronic heart failure (Peppel et al., 1997). However, the deleterious effects of enhanced β-adrenoceptor signaling on myocardial function might be due to the fact that in these mice, the components of the β-adrenoceptor system (β_1- and β_2-adrenoceptors and $G_{s\alpha}$) have been overexpressed at high levels. On the other hand, limited mild overexpression might have beneficial effects: Dorn et al. (1999) have recently shown that in the murine model of cardiac failure due to cardiac-directed $G_{q\alpha}$ overexpression (D'Angelo et al., 1997), concomitant overexpression of β_2-adrenoceptors at low expression levels (about 30-fold) rescued ventricular function, whereas overexpression of β_2-adrenoceptors at higher levels (140- to 1000-fold) worsened heart failure.

In addition to β-adrenoceptors and $G_{s\alpha}$ activity, the activity of GRK2 appears to have a pivotal role in the regulation of cardiac function. The fact that in mouse heart overexpressing GRK2 the functional responsiveness to β_1-adrenoceptor stimulation is decreased is in favor of the idea that in vivo β_1-adrenoceptors are a substrate for GRK2. In addition, as discussed above, in the mouse heart overexpressing the GRK2 inhibitor, basal in vivo contractility is enhanced, which suggests that GRK2 may exert tonic inhibition of cardiac β_1-adrenoceptors. Thus, all data published so far suggest that a decrease in the activity of cardiac GRK2 might be favorable for cardiac performance.

As mentioned above, constant stimulation of the cardiac β-adrenoceptor/G protein(s)/adenylyl cyclase system (as in β-adrenoceptor- or $G_{s\alpha}$-overexpressing mice) leads over time to symptoms of heart failure. On the other hand, it has been shown that in cardiomyocytes, the molar proportion of the components of the β-adrenoceptor/G_s-protein/adenylyl cyclase system is approximately 1:230:3 (Alousi et al., 1991; Post et al., 1995). From this stoichiometry, it appears that the level of adenylyl cyclase expression might be the critical determinant for increases in cAMP (which mediate contractile responses to β-adrenoceptor stimulation; see *Presence and Function of Receptor Subtypes in Human Heart. C1. β_1- and β_2-Adrenoceptors*). In fact, a recent study in isolated rat ventricular cardiomyocytes revealed a proportional β-adrenoceptor-mediated increase in cAMP formation with increased overexpression of adenylyl cyclase type VI (a major adenylyl cyclase isoform in cardiac myocytes; Ishikawa and Homcy, 1997; Ping et al., 1997), whereas neither β-adrenoceptor number nor the immunodetectable amount of G_s or G_i protein was changed (Gao et al., 1998). In addition, cardiac overexpression of adenylyl cyclase type VI in transgenic mice resulted in an enhanced cardiac function and in increased cAMP production in cardiomyocytes after β-adrenoceptor stimulation but in unchanged basal cAMP levels and cardiac function (Gao et al., 1999); again, β-adrenoceptor number and the amount of G_s and G_i protein were unchanged, whereas that of GRK2 was increased about 2-fold. These mice did not exhibit any signs of myocardial fibrosis, in contrast to the β-adrenoceptor- or $G_{s\alpha}$-overexpressing mice (see above). Cardiac overexpression of adenylyl cyclase type VI might be, therefore, an alternative approach to improve cardiac function in chronic heart failure; it appears to have the advantage that β-adrenoceptor signaling (which is continuously activated in β-adrenoceptor- and $G_{s\alpha}$-overexpressing mice) is in adenylyl cyclase-overexpressing mice enhanced only when β-adrenoceptors are stimulated and not in the resting state. In fact, a very recent study demonstrates that in mice with cardiomyopathy caused by cardiac-directed $G_{q\alpha}$ overexpression [that is accompanied by decreased cardiac responsiveness and cAMP formation to β-adrenoceptor stimulation (D'Angelo et al., 1997)], additional expression of adenylyl cyclase type VI restored β-adrenoceptor responsiveness and improved cardiac function (Roth et al., 1999).

V. Receptor Polymorphisms

In recent years, there has been an amazing proliferation of identified adrenoceptor subtypes, particularly when splice variants for some of the receptors are taken into account. In addition, polymorphisms have been identified for several adrenoceptor genes (Büscher et al., 1999). Although the functional role of polymorphisms of α_1- and α_2-adrenoceptor subtypes for cardiac physiology remains unclear, polymorphisms of β_2-adrenoceptors may be more relevant in this context.

In a series of elegant studies, Liggett and coworkers recently demonstrated four polymorphisms in the coding block of the gene encoding the β_2-adrenoceptor resulting in changes of amino acids: the most common polymorphism occurred at position 16, where arginine is replaced by glycine (Arg16→Gly), and in the homozygous form it makes up to 50% of the β_2-adrenoceptor in the

normal population. Additional polymorphisms were found at position 27 (glutamine is substituted by glutamic acid, Gln27→Glu, which represents 25% in the homozygous form in the normal population), at position 34 (valin is replaced by methionin, Val34→Met, which is less than 1% in the normal population), and at position 164 (threonine is replaced by isoleucine, Thr164→Ile, which was found in approximately 6% of the normal population in the heterozygous state; for a review, see Liggett, 1995; Fig. 11). To study the functional consequence of these polymorphisms, each of the β_2-adrenoceptor variants (produced by site-directed mutagenesis of the WT β_2-adrenoceptor, as originally described by Kobilka et al., 1987) was expressed in Chinese hamster fibroblast (CHW) cells, and their properties were studied. The Thr164→Ile mutant exhibited decreased affinities for the agonists isoprenaline, adrenaline, and noradrenaline and about 50% reduced maximal adenylyl cyclase stimulation in response to adrenaline and showed marked uncoupling from G_s (Green et al., 1993). Moreover, when overexpressed in the heart of transgenic mice (Turki et al., 1996), this mutant showed a significantly reduced basal adenylyl cyclase activity in myocytes compared with transgenic mice overexpressing the WT β_2-adrenoceptor; the same held true for maximal isoprenaline-stimulated adenylyl cyclase. In addition, in intact animals, resting heart rate and the heart rate response to isoprenaline were reduced compared with transgenic mice overexpressing the WT β_2-adrenoceptor. In contrast to the Thr164→Ile mutation, the Arg16→Gly and Gln27→Glu mutations expressed in the CHW cells exhibited normal agonist and antagonist binding affinities and normal adenylyl cyclase activation (Green et al., 1994). However, these two mutants differed significantly in their susceptibility to agonist-induced down-regulation. WT β_2-adrenoceptors (with Arg16 and Gln27) were down-regulated by 26% after a 24-h incubation with isoprenaline, whereas the Gly16 mutant was down-regulated by 41% and the Glu27 mutant did not show significant down-regulation. Cells with coexpression of the Gly16 and Glu27 showed a 39% down-regulation (i.e., similar to the Gly16 mutant; Green et al., 1994). Very similar results were also obtained in primary cell lines of human bronchial smooth muscles (obtained at autopsy) natively expressing β_2-adrenoceptor polymorphisms: the Arg16→Gly polymorphism exhibited a markedly higher degree of agonist-induced down-regulation and functional desensitization than the Gln27→Glu polymorphism (Green et al., 1995). Thus, it appears that the NH_2-terminal polymorphisms of the β_2-adrenoceptor are linked to receptors with different susceptibilities to agonist-induced down-regulation. Recently, McGraw et al. (1998) identified a polymorphism of the 5′ leader cistron of the human β_2-adrenoceptor; this polymorphism was shown to regulate cellular expression of the two common β_2-adrenoceptor polymorphisms Arg16→Gly and Gln27→Glu. In addition, Scott et al. (1999) described a total of eight polymorphisms in the promotor region of the β_2-adrenoceptor gene, which in preliminary studies appear to alter expression of a

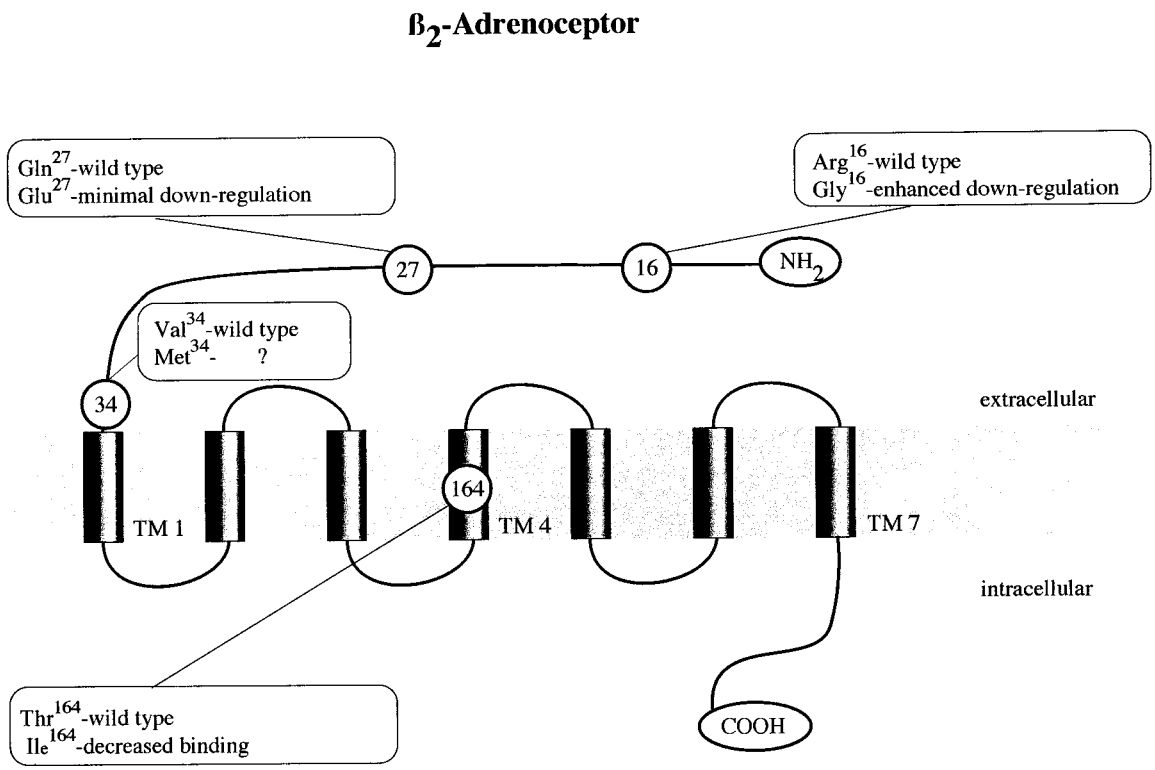

FIG 11. β_2-Adrenoceptor polymorphisms. Adapted from Büscher et al. (1999) with some modifications.

luciferase-based reporter plasmid in COS-7 cells. Luciferase constructs containing the two most frequent haplotypes within 549 bp of 5′ flanking DNA were transiently transfected into COS-7 cells. Luciferase activity was significantly reduced in cells transfected with the mutant constructs versus wild-type constructs, suggesting a potential alterations of β_2-adrenoceptor gene expression.

Studies have been performed to find out whether the Arg16→Gly and/or Gln27→Glu polymorphism might be markers of different disease states, such as asthma or hypertension. In general, the findings do not support the concept that the polymorphisms are the cause of asthma, but they may significantly modify the course and severity of the disease (for recent reviews, see Lipworth, 1998; Büscher et al., 1999).

One recent study investigated a possible role of the β_2-adrenoceptor polymorphisms in patients with chronic heart failure (Liggett et al., 1998). It was found that there was no difference in the allele frequencies in 259 patients with congestive heart failure and in 212 healthy controls. However, patients heterozygous for the Ile164 polymorphism had a significantly reduced survival rate (death or cardiac transplantation) compared with patients with the WT Thr164 β_2-adrenoceptor (Fig. 12). In addition, there was a weak effect of the Gly16 and Gln27 polymorphism if survival rate is determined at a midpoint. These results indicate that patients harboring the Ile164 mutant of the β_2-adrenoceptor are candidates for very early intensive treatment and/or cardiac transplantation.

Very recently, two polymorphisms for the β_1-adrenoceptor were found in humans: at position 49, serine is substituted for glycine, and at position 389, glycine is substituted for arginine (Maqbool et al., 1999; Mason et al., 1999; Tesson et al., 1999). The functional relevance of these polymorphisms, however, is not known at the present. Also, for the β_3-adrenoceptor, a polymorphism has been found in humans: tryptophan at position 64 is substituted by arginine (Trp64→Arg; Clement et al., 1995; Walston et al., 1995; Widen et al., 1995). However, because it is rather unclear whether the β_3-adrenoceptor exists in the human heart (see *Is There a Third (or Fourth) β-Adrenoceptor Subtype Present in Human Heart?*), it is beyond of scope of this article to discuss the possible role of this genetic variant for earlier onset of non-insulin-dependent diabetes mellitus or obesity. The reader is referred to a recent review by Strosberg (1997b). Future research into the consequences of receptor polymorphisms for cardiovascular function may not only further improve our pathophysiological understanding but also help to identify subsets of patients who might benefit from specific therapeutic strategies.

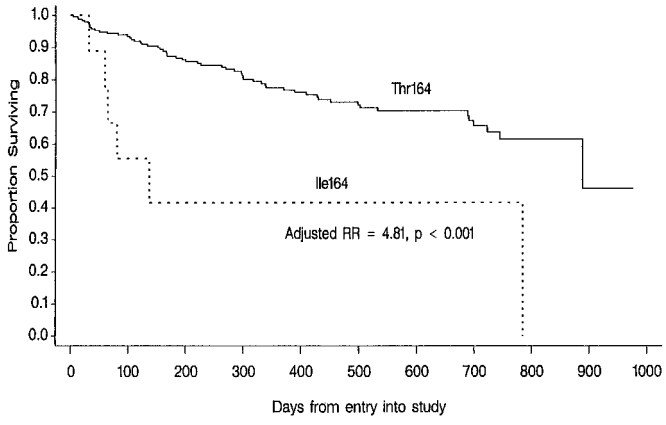

FIG 12. Kaplan-Meier survival curves for patients with congestive heart failure harboring the WT β_2-adrenoceptor or the Ile164 polymorphism. The Ile164 polymorphism was found only in the heterozygous state. The survival function is the proportion of patients who have not died or have not undergone cardiac transplantation. As is shown, individuals with the Ile164 polymorphism (n = 10 patients) had an increased risk of death or transplantation compared with those with the Thr164 (WT) receptor (n = 247 patients). From Liggett et al. (1998).

VI. Conclusions

The pivotal role of the sympathetic and parasympathetic systems in the control of cardiac function has been known for many decades. However, identification of the specific receptors and signal transduction components involved in this control at the molecular level has become possible only due to the progress of molecular pharmacology and recombinant DNA technology in the past 20 years. The application of these advances to the human heart has been hampered by the limited access of healthy tissues for in vitro studies and by the ethical and technical constraints for in vivo studies. Nevertheless, marked progress has been achieved in this field. Combinations of in vitro and in vivo studies have demonstrated many expected similarities between human and other mammalian hearts but also have highlighted several features that may be unique for the human heart.

Many important physiological effects, such as aging and disease states, such as congestive heart failure develop chronically over many years. Although impressive progress has been made in the understanding of short-term regulation of cardiac function (i.e., chronotropy and inotropy) by the autonomic nervous system, much less is known regarding the role of chronic alterations in autonomic input. Because such chronic alterations may have a major impact on human health and disease management, a major challenge of studies on the autonomic regulation of human cardiac function in the forthcoming years will be the understanding of slowly developing and chronic processes such as hypertrophy or catecholamine toxicity.

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