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β_1- and β_2-Adrenoceptors in the Human Heart: Properties, Function, and Alterations in Chronic Heart Failure*

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I. Introduction

THE endogenous catecholamines, noradrenaline and adrenaline, exert their differential biological effects through activation of specific membrane-bound receptors. Ahlquist (1948), on the basis of the different relative potencies of a series of agonists, first suggested a subdivision of ARs‡ into the subtypes α and β. However, it eventually became apparent that this subclassification was not sufficient to explain the manifold effects of catecholamines observed in physiological and pharmacological studies. Thus, two subtypes of α- and β-ARs were subsequently defined and termed α_1/α_2 (Langer, 1974; Berthelsen and Pettinger, 1977; Starke and Langer, 1979) and β_1/β_2 (Lands et al., 1967a,b). However, it now seems to be necessary to further subdivide these subtypes; thus, at present three α_1-AR subtypes (α_{1A}, α_{1B}, and α_{1C}), three α_2-AR subtypes (α_{2A} = α_2-C10, α_{2B} = α_2-C2, and α_2-C4), and three β-AR subtypes (β_1, β_2, and β_3) have been cloned (see Watson and Abbott, 1991). Whether all of these subtypes have a functional importance, however, remains to be elucidated.

The original subclassification of β-ARs into β_1 in the heart (where noradrenaline and adrenaline are equally potent) and β_2 in vascular and bronchial smooth muscles (where adrenaline is about 10- to 30-fold more potent than noradrenaline) presumes a high degree of organ specificity of the β-AR subtypes (Lands et al., 1967a,b). This hypothesis has now evolved (mainly based on data derived from radioligand-binding studies) into the concept that in a variety of organs, including the heart, both β_1- and β_2-ARs coexist, although often one subtype predominates. This was first suggested in 1972 by Carlsson et al. who showed that equal submaximal chronotropic responses of the cat heart to β-AR agonists were antagonized to different extents by the β_1-AR-selective antagonist practolol and by the β_2-AR-selective antagonist H 35/25. Similar effects have been reported in numerous in vivo as well as in vitro experiments in cat, dog, and guinea pig hearts as well as in guinea pig and rabbit trachea, thus supporting the concept of the coexistence of β_1- and β_2-ARs in a single organ (for reviews, see Daly and Levy, 1979; Minneman et al., 1981; Stiles et al., 1984; Brodde, 1989).

It is now possible to determine the relative proportion of β_1- and β_2-ARs present in a tissue using radioligand-binding studies; frequently used β-AR antagonist radioligands are listed in table 1. The technique most widely applied to quantify the relative proportion of β_1- and β_2-ARs is to assess inhibition of binding of nonselective β-AR radioligands (that label β_1- and β_2-ARs with similar affinity; see table 1) by unlabeled antagonists that have shown in vitro selectivity for β_1-AR [e.g., atenolol (Barrett et al., 1973; Barrett, 1977), betaxolol (Boudot et al., 1979), bisoprolol (Schliep and Harting, 1984; Wang et al., 1985; Kaumann and Lemoine, 1985; Brodde, 1986), CGP 20712 A (Dooley et al., 1986), ICI 89,406 (Svendsen et al., 1979; Engel et al., 1982), and LK 204545 (Milavec-Krizman et al., 1985)] or β_2-AR [e.g., ICI 118,551 (Bilski et al., 1983; Lemoine et al., 1985)]. The resulting competition curves are shallow or biphasic, if both β-AR subtypes are present in the tissues (fig. 1), whereas steep and monophasic competition curves are obtained if only one β-AR subtype is present. On the other hand, nonselective β-AR antagonists always inhibit binding with monophasic competition curves independently of whether one or two subtypes are present. Three different analytical methods have been commonly used to analyze such competition curves: (*a*) graphical analysis of the curves by transformation of the binding data into a modified Scatchard or Hofstee plot (Barnett et al., 1978; Rugg et al., 1978); (*b*) iterative computer analysis of the Hofstee plots (Minneman et al., 1979); and (*c*) analysis of nontransformed binding data with a nonlinear least-squares computer modeling method (De Lean et al., 1982). Computer programs are now commercially available (e.g., LIGAND, Biosoft, Cambridge, United Kingdom; InPlot, GraphPad Software, San Diego, CA; Sigmaplot, Sigma, St. Louis, MO) which perform iterative nonlinear least-squares fitting of the nontransformed data.

An alternative approach for the direct characterization of *one* β-AR subtype in tissues containing both β_1- and β_2-ARs is the use of a radioligand highly selective for that subtype (fig. 1). The advantage of this approach is that it allows the *direct* determination of one β-AR subtype as well as the *direct* determination of the true affinity of β-AR drugs for that subtype without interference by the other one. At least two selective β_1-AR radioligands are available at present (see table 1): [^{3}H] bisoprolol (Wang et al., 1985; Kaumann and Lemoine, 1985) and [^{3}H]CGP 26505, the (−)-form of the highly selective β_1-AR antagonist, CGP 20712 A; for *direct* β_2-AR labeling, [^{3}H]-ICI 118,551 is available (Lemoine et al., 1985).

The coexistence of β_1- and β_2-ARs has been demonstrated with the technique of radioligand binding in the hearts of rats, cats, guinea pigs, dogs, and rabbits (for references, see Minneman et al., 1981; Stiles et al., 1984;

‡ Abbreviations: AR, adrenoceptor; ICYP, iodocyanopindolol; CEB, calcium entry blocker; ACE, angiotensin-converting enzyme; cAMP, cyclic 3′,5′-adenosine monophosphate; AC, adenylate cyclase; MVD, mitral valve disease; PDE, phosphodiesterase; G_s, stimulatory guanine nucleotide-binding protein; G_i, inhibitory guanine nucleotide-binding protein; $G_{s\alpha}$, α subunit of G_s; NYHA, New York Heart Association; HOCM, hypertrophic obstructive cardiomyopathy; P/V, systolic blood pressure/end-systolic left ventricular volume ratio; TF, tetralogy of Fallot; DCM, dilated cardiomyopathy; GTP, guanosine 5′-triphosphate; ADP, adenosine 5′-diphosphate; ICM, ischemic cardiomyopathy; DHA, dihydroalprenolol; AVD, aortic valve disease; PAA, partial agonistic activity; B_{max}, maximal number of binding sites; pD_2, −log of concentrations (M) causing 50% of maximal response; pEC_{50}, −log EC_{50} (M); pK_B, −log equilibrium dissociation constant (M).

TABLE 1
β-Adrenoceptor antagonist radioligands

Radioligands	Maximum specific radioactivity (Ci/mmol)	Approximate K_D (pM)	Selectivity	References
(−)-[^{3}H]DHA	110	500–2000	2.3-fold β_2-AR selective	Neve et al. (1986)
(−)-[^{3}H]Bupranolol	18	300–1600	2- to 6-fold β_2-AR selective	Lemoine et al. (1985) Kaumann and Lemoine (1987)
(−)-[^{3}H]CGP 12177	60	300–2500	2.7-fold β_1-AR selective	Nanoff et al. (1987)
(−)-[^{3}H]Bisoprolol	40	4700–7000	70- to 100-fold β_1-AR selective	Kaumann and Lemoine (1985) Brodde (1986)
[(−)-form of CGP 20712A] [^{3}H]CGP 26505 A	29	580	1000-fold β_1-AR selective (at least)	
(±)-[^{3}H]ICI 118,551	21	400–600	200- to 300-fold β_2-AR selective	Lemoine et al. (1985)
(±)-[^{125}I]Iodohydroxybenzylpindolol	2200	10–1000	5.8-fold β_2-AR selective	Neve et al. (1986)
(−)-[^{125}I]ICYP	2200	5–50	2-fold β_2-AR selective	Neve et al. (1986)
(−)-[^{125}I]Iodopindolol	2200	20–90	3.2-fold β_2-AR selective	Neve et al. (1986)

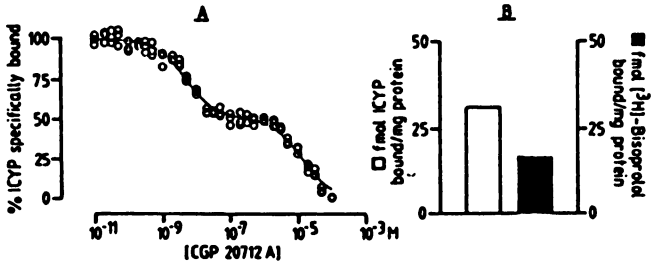

FIG. 1. Determination of β_1- and β_2-ARs in human left ventricular membranes. A, Inhibition of ICYP binding by the selective β_1-AR antagonist CGP 20712 A. The resulting concentration-inhibition curve is analyzed by a computer-assisted iterative curve-fitting program to yield the relative amount of β_1- (high-affinity component) and β_2-ARs (low-affinity component). B, Determination of the total amount of β-ARs by Scatchard (1949) analysis of binding of the nonselective radioligand ICYP and simultaneous determination of the amount of β_1-ARs by Scatchard (1949) analysis of binding of the selective β_1-AR radioligand (−)-[^{3}H]bisoprolol. Note that with both approaches a nearly identical β_1-AR to β_2-AR ratio of approximately 60:40% is reached. A typical experiment in left ventricular membranes obtained from a patient with end-stage DCM is shown. From the unpublished data of O.-E. Brodde.

Summers et al., 1987; Brodde, 1987, 1989). As a general rule, β_2-ARs are present in larger amounts in atria than in ventricles. However, the physiological role of these cardiac β_2-ARs is still not completely understood. Although β_2-ARs are involved in the positive *chronotropic* effects of β-AR agonists in right atria from rats, guinea pigs, cats, and dogs (Carlsson et al. 1972, 1977; Dreyer and Offermeier, 1975; Yabuuchi, 1977; Yabuuchi et al., 1977; Johansson and Persson, 1983; Kaumann and Lemoine, 1985; Lemoine et al. 1985; Liang et al., 1985; O'Donnell and Wanstall, 1985; Kaumann, 1986; Molenaar and Summers, 1987) but not rabbits (Costin et al., 1983; Wilson and Lincoln, 1984; Tenner et al., 1989), it is still a matter of controversy whether β_2-ARs can contribute to the positive *inotropic* effects of β-AR agonists in atrial and ventricular preparations from these species (Yabuuchi, 1977; O'Donnell and Wanstall, 1979; Bryan et al., 1981; Broadley and Hawthorn, 1983; Costin et al., 1983; Freyss-Beguin et al., 1983; Kaumann et al., 1983; Kenakin and Beek, 1984; Wilson and Lincoln, 1984; Juberg et al., 1985; Kaumann and Lemoine, 1985; Lemoine et al., 1985; Molenaar and Summers, 1987; Tenner et al., 1989; Yanagisawa et al., 1989).

The first evidence that a heterogeneous population of β_1- and β_2-ARs also might exist in human myocardium was presented in 1974 by Åblad et al. These authors, using the isolated electrically driven human right atrium, found that the β_1-AR-selective antagonist H 93/26 (metoprolol) was more potent in antagonizing the positive inotropic effect of noradrenaline than it was that of adrenaline. In contrast the nonselective β-AR antagonist, propranolol, inhibited responses to both catecholamines to about the same degree. Similar results were reported by Bonelli (1978) who observed that in humans the β-AR antagonist mepindolol inhibited the positive chronotropic effect of isoprenaline much more effectively than it did isoprenaline's positive inotropic effect.

During the last 9 years β_1- and β_2-ARs have been directly identified in human atrial and ventricular tissues by radioligand-binding studies. In this article, the properties, distribution, and regulation of the human cardiac β-AR subtypes and their implications for therapeutic interventions will be discussed.

II. β_1- and β_2-Adrenoceptors in the Nonfailing Human Heart

A. Distribution

The first direct demonstration of β_1- and β_2-ARs in human right atrial (Taton et al., 1982; Brodde et al., 1982) and left ventricular tissue (Stiles et al., 1982; Brodde et al., 1982) was reported in 1982 and subsequently confirmed by many groups (for recent reviews, see Brodde, 1987; Jones et al., 1989; Feldman and Bristow, 1990; Bristow et al., 1990). However, because human cardiac tissue is available only during cardiac surgical

procedures from patients with a variety of cardiac diseases and because heart failure affects cardiac β-AR and β-AR subtypes (see section III), the reported data concerning β-AR density and β-AR subtype distribution vary markedly. This section deals with data regarding *nonfailing* hearts to try to give a picture of β_1- and β_2-AR distribution in the normal human heart.

Most available data concerning β-ARs in nonfailing human hearts are obtained in left and right ventricular free walls from would-be cardiac transplant donors whose hearts could not be transplanted for technical reasons. However, it should be kept in mind that the main organ donors are patients with severe head injury in whom endogenous catecholamines are markedly elevated, possibly leading to desensitization (and down-regulation) of cardiac β-ARs; in addition, many of these patients need catecholaminergic support (Darby et al., 1989). Four groups (Bristow et al., 1986, 1991; Böhm et al., 1989b; Brodde et al., 1989b,c; Steinfath et al., 1991a,b) have reported that the amount of left and right ventricular β_2-ARs is approximately 20% of the total β-AR population (table 2). These data were obtained independently of whether (−)-[^{125}I]ICYP or [^{3}H]CGP 12177 was used as the radioligand and of whether binding was performed in a washed 50,000 × *g* fraction after extraction of the contractile proteins with KCl or in a crude membrane preparation. Moreover, in both right and left ventricular membranes the mean number of β-ARs was approximately 70 to 100 fmol/mg protein (table 2).

Similar data (approximately 79 fmol/mg protein) have been reported for the left ventricle obtained at autopsy within ½ hour of death (Stiles et al., 1983). In contrast, in hearts "obtained soon after death" (elapsed time not specified) left ventricular β-AR levels were similar (approximately 76 fmol/mg protein), but the proportion of β_2-ARs appeared to be higher ((β_1-AR to β_2-AR ratio, 65:35%) (Heitz et al., 1983; cf. table 2).

For assessment of β-AR density and β_1- and β_2-AR distribution in right atria, many studies have been performed on right atrial appendages that are routinely excised when starting extracorporeal circulation in patients undergoing an elective coronary artery bypass grafting operation. Because these patients generally do not have any significant signs of heart failure, they can

TABLE 2

Numbers of β-AR and subtype distribution in the nonfailing human heart

Tissue and ligand	B_{max} (fmol/mg protein)	β_1-AR to β_2-AR ratio (%)	Tissue source*	References
Right atrium				
ICYP	99 ± 8 (4)†	ND‡	A	Böhm et al. (1991)
ICYP	78 ± 7 (6)	71:29	A	M. Steinfath, personal communication
ICYP	84 ± 14 (3)	74:26	A, B	Stiles et al. (1983)
ICYP	79 ± 4 (44)	69:31	D	Brodde et al.§
Left atrium				
ICYP	111 ± 6 (4)	ND	A	Böhm et al. (1991)
ICYP	82 ± 7 (6)	69:31	A	M. Steinfath, personal communication
ICYP	44 ± 5 (4)	63:37	C	Heitz et al. (1983)
Right ventricle				
ICYP	79 ± 13 (4)	77:23	A	Bristow et al. (1986)
ICYP	97 ± 7 (20)	82:18	A	Bristow et al. (1991)
ICYP	66 ± 4 (4)	ND	A	Böhm et al. (1991)
ICYP	72 ± 7 (7)	80:20	A	M. Steinfath, personal communication
ICYP	74 ± 12 (3)	79:21	E	Brodde et al. (1989b)
Left ventricle				
ICYP	88 ± 7 (12)	77:23	A	Bristow et al. (1986)
ICYP	88 ± 6 (20)	76:24	A	Bristow et al. (1991)
[^{3}H]CGP 12177	93 ± 4 (3)	79:21	A	Böhm et al. (1989b)
ICYP	84 ± 4 (5)	ND	A	Böhm et al. (1991)
ICYP	70 ± 6 (7)	79:21	A	Steinfath et al. (1991a, b)
ICYP	66 ± 9 (4)	78:22	A	Brodde et al. (1989c)
ICYP	79 ± 3 (3)	86:14	A, B	Stiles et al. (1983)
ICYP	76 ± 8 (4)	65:35	C	Heitz et al. (1983)

* A, obtained from would-be cardiac transplant donors; B, obtained at autopsy within ½ h of death; C, obtained "soon after death" (elapsed time not specified); D, obtained during elective coronary artery bypass grafting from patients without apparent heart failure, who were treated with only nitrates, if at all; E, obtained from two patients with acyanotic TF ("pink Fallot") with minimal right to left shunt (NYHA class I to II) and one patient with residual ventricular septal defect (NYHA class I).

† Mean ± SEM (number of experiments, performed on different hearts).

‡ Not determined.

§ Data recalculated from Brodde et al. (1986a, 1989c), Michel et al. (1988), Motomura et al. (1990a), and Brown et al. (1991).

be taken as "nearly normal." The proportion of β_2-ARs in this tissue, however, varied markedly, ranging from 18 to 55% (Brodde et al., 1983; Robberecht et al., 1983; Hedberg et al., 1985; for additional references, see Brodde, 1987; Jones et al., 1989). The reason for this variability is not known but may be due to different methods of transporting the tissue to the laboratory, the use of different radioligands, and/or differential techniques in preparing the membrane fractions or may reflect prior drug administration (see section VI). Another possibility, as illustrated in rat and rabbit lung membranes, is that, after solubilization, β_1-ARs are much more labile than β_2-ARs (Dickinson and Nahorski, 1981). A similar instability of β_1-ARs has been described for human right atrial tissue (Stiles et al., 1983). Thus, a longer storage of this tissue (especially at not appropriately low temperatures) may result in a relative loss of β_1-ARs, thereby apparently increasing the relative amount of β_2-ARs. Studies conducted with fresh tissue show good agreement. In our own studies of 44 patients treated only with nitrates (if at all), but not with β-AR antagonists, calcium antagonists (CEBs), and ACE inhibitors, we found a B_{max} of 79 fmol/mg protein and a β_1-AR to β_2-AR ratio of 69:31% (table 2). These data are in good agreement with the early data of Stiles et al. (1983) who found, in right atria from one would-be cardiac transplant donor and from two autopsies within ½ hour of death, a B_{max} of 84 fmol/mg protein and a β_1-AR to β_2-AR ratio of 74:26% (table 2).

Very recently, two studies of right atria of nonfailing hearts from would-be cardiac transplant donors were performed. The data fit quite well with those given above: the B_{max} amounted to 78 to 99 fmol/mg protein and the β_1-AR to β_2-AR ratio was 71:29% (Böhm et al., 1991; M. Steinfath, personal communication, cf. table 2).

Only three studies of β-AR subtype distribution in nonfailing human left atria have been published. In hearts "obtained soon after death" (elapsed time not specified), B_{max} was 44 fmol/mg protein and the β_1-AR to β_2-AR ratio was 63:37% (Heitz et al., 1983; table 2). On the other hand, in left atria of nonfailing hearts from would-be cardiac transplant donors, a B_{max} of 82 to 111 fmol/mg protein and a β_1-AR to β_2-AR ratio of 69:31% (Böhm et al., 1991; M. Steinfath, personal communication, cf. table 2) was found.

It is still an open question whether both β_1- and β_2-ARs in the human heart are located on the same cell or on different cell types, as has been shown for many animal species. Thus, in rat and guinea pig hearts, β_2-ARs are located on coronary endothelial cells (Freissmuth et al., 1986) and fibroblasts (Lau et al., 1980). Although in the heart β_1- and β_2-ARs are found on myocardial and conducting tissues (Summers et al., 1987), β_2-ARs are also associated with cardiac nerves (Molenaar et al., 1987, 1988), epicardial tissue (Molenaar et al., 1987), and blood vessels (Freissmuth et al., 1986; Molenaar et al., 1987, 1988).

For the following reasons it is not unlikely that in the human hearts both β_1- and β_2-ARs are located on cardiomyocytes: (*a*) Autoradiographic studies have shown that, in human right atrial appendages, left atrial free wall and left papillary muscle β_1- and β_2-ARs are evenly distributed over myocytes; these tissues also have a lower density of β_2-ARs in the intimal surface of their small intramyocardial blood vessels (Buxton et al., 1987; Summers et al., 1989). Recent studies using ICYP binding (Steinfath et al., 1991c) showed that in human left ventricular myocardium of nonfailing hearts the number of β-ARs and the relative proportion of β_1- and β_2-ARs do not vary within different regions (papillary muscle, free wall, ventricular apex, and interventricular septum). In addition, it has recently been shown that in human epicardial coronary arteries only β_1-ARs mediate the catecholamine-induced relaxation (Toda and Okamura, 1990); in human right and left coronary arteries, recent autoradiographic findings show that β_1-ARs predominate (Amenta et al., 1991). It is also evident that β_1-ARs predominate in the tunica media (probably localized within the arterial smooth muscle), whereas β_2-ARs predominate in the tunica adventitia, in the adventitia-media border, and in the tunica intima (Amenta et al., 1991). However, it should be emphasized that selective β_1-AR-mediated relaxation of coronary arteries is only true for large arteries of the type amenable to organ bath or autoradiographic studies. Animal studies of the coronary circulation in vivo have demonstrated that there is a highly significant β_2-AR-mediated component of dilation, which resides on the small resistance vessels (Feigl, 1983; Vatner et al., 1986; Young and Vatner, 1986). (*b*) As will be discussed, in the human heart both β_1- and β_2-ARs contribute to positive inotropic effects of β-AR agonists.

B. Coupling to Adenylate Cyclase

Since the original observation of Rall and Sutherland (1958) that in the heart the formation of cAMP is catalyzed by adrenaline, numerous studies have shown that cAMP plays an important role in the regulation of metabolism and function in the heart. It is now generally accepted that cAMP is the "second messenger" for the positive inotropic effects induced by β-AR stimulation (Tsien, 1977; Scholz, 1980). It is, therefore, not surprising that also in the human heart β-ARs couple to AC.

Meinertz et al. (1974) were the first to demonstrate that dibutyryl cAMP causes positive inotropic effects in the isolated human left ventricular myocardium. The first reports demonstrating that, in ventricular membrane preparations obtained from nonfailing hearts or from patients with MVD, isoprenaline and noradrenaline activate AC appeared in 1982 (Bristow et al., 1982; Kaumann et al., 1982). Subsequently, it was shown that catecholamines activated AC in right atrial membranes

obtained from patients undergoing elective coronary artery bypass grafting with an order of potency: isoprenaline > adrenaline ≥ noradrenaline (Brodde et al., 1983), indicating a predominance of β_1-ARs (Lands et al., 1967a,b). In contrast, studies of human right atrial membranes using isoprenaline, several β_2-AR agonists (procaterol, zinterol, salbutamol, and fenoterol), and β_1-AR antagonists (metoprolol, practolol, atenolol) indicated that only β_2-AR stimulation was involved in the activation of AC (Waelbroeck et al., 1983). Subsequently, it was shown that in membranes from human right atria the β_2-AR-selective agonist procaterol caused AC stimulation that amounted to approximately 70% of that produced by isoprenaline, although only 30% of the total β-AR population is of the β_2 subtype (Brodde et al., 1984; cf. table 2). This suggested that in the human heart β_2-ARs are more efficiently coupled to AC than are β_1-ARs. This was subsequently confirmed in several elegant studies by Kaumann and associates who, using propranolol (Gille et al., 1985), ICI 118,551 (Kaumann and Lemoine, 1987; Lemoine et al., 1988), CGP 20712 A (Kaumann and Lemoine, 1987), and atenolol (Lemoine et al., 1988) as antagonists, clearly demonstrated that in human right atrial and left ventricular membranes the catecholamines adrenaline and noradrenaline activate AC predominantly (60 to 70%) via β_2-ARs and only to a minor extent via β_1-AR stimulation (fig. 2), although in both tissues β_1-ARs predominate. Similarly, isoprenaline was found to activate right atrial (Bjornerheim et al., 1990) and ventricular (Bristow et al., 1989) AC mainly through β_2-AR stimulation (fig. 2).

Thus, in the human heart the anomaly exists that AC is preferentially activated by β_2-AR stimulation, although β_1-ARs predominate. In cats, for example, the fractional stimulation of AC matches the relative amount of β_2-ARs (Kaumann and Lemoine, 1985; Kaumann et al., 1989b). The reason for this unusual behavior is not known at present. A possible reason for this species difference may be that human β_1-ARs are partially uncoupled from the AC due to (perioperative) high plasma noradrenaline levels and high sympathetic drive, because all of these studies have been performed in hearts from patients undergoing open heart surgery with more or less established heart failure. However, this seems not to be the case, because it recently was shown (Bristow et al., 1989) that in ventricular membranes from normal hearts the isoprenaline-induced activation of AC is predominantly mediated by β_2-ARs (fig. 2), although in the same membrane fraction only 20% of the β-ARs were of the β_2 subtype (cf. table 2). Thus, the mechanism underlying the different coupling efficiencies of human cardiac β_1- and β_2-ARs to AC remains to be elucidated.

Isoprenaline caused an increased formation of cAMP not only in broken cell preparations but also in intact human right atria. In the isolated, electrically driven right atrial strip, a submaximal positive inotropic concentration of isoprenaline (300 nM) produced a rapid increase in the intracellular level of cAMP (fig. 3). This was maximal after 60 s, whereas the increase in contractile force reached its maximum after 120 to 240 s (Ikezono et al., 1987). In the presence of the PDE inhibitor, papaverine, both the increase in cAMP and positive inotropic effect of isoprenaline were enhanced, whereas the β-AR antagonist propranolol attenuated both effects. Thus, the four important criteria for a second messenger role of cAMP in the positive inotropic effects of β-AR agonists on human heart seem to be fulfilled (Sutherland et al., 1968): (*a*) in broken cell preparations AC responds to the same agents that are effective in intact tissues. Furthermore, in both experimental systems the order of potency of agonists is the same and the effectiveness of competitive antagonists is similar; (*b*) the increase in cAMP precedes the physiological response; (*c*) inhibition of the breakdown of cAMP by PDE inhibitors enhances the effects of agonists on both cAMP and force of contraction; and (*d*) dibutyryl cAMP mimics the effects of drugs or hormones that activate AC.

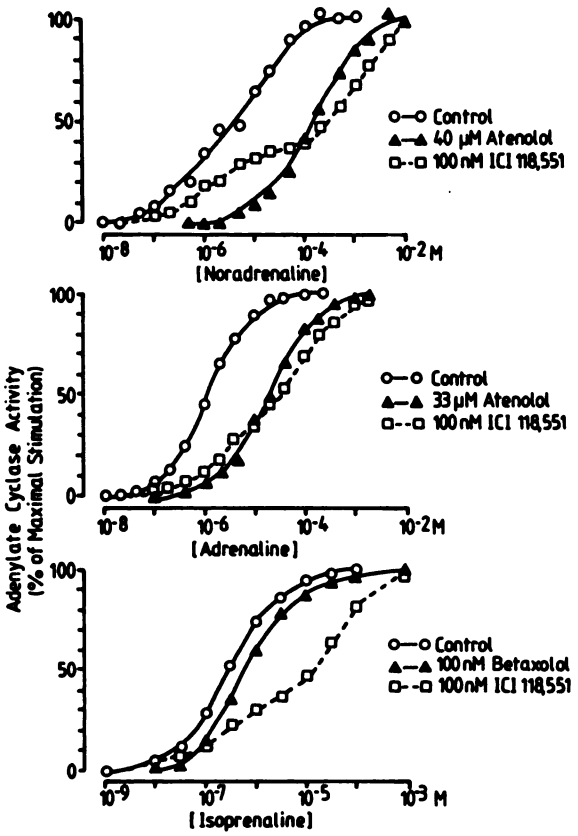

FIG. 2. Antagonism of noradrenaline-induced (top), adrenaline-induced (middle), and isoprenaline-induced (bottom) stimulation of human left ventricular AC by β_1- (atenolol or betaxolol) and β_2- (ICI 118,551) AR-selective antagonists. For noradrenaline and adrenaline experiments, left ventricles were from patients with MVD; for isoprenaline experiments, the ventricles were from nonfailing hearts. Note that for all three agonists approximately 60 to 70% of maximal AC activity was mediated through β_2-ARs (ICI 118,551 sensitive), although β_1-ARs predominate in ventricular membranes (cf. table 2). From Lemoine et al. (1988; noradrenaline and adrenaline data) and Bristow et al., (1989; isoprenaline data) and used with permission.

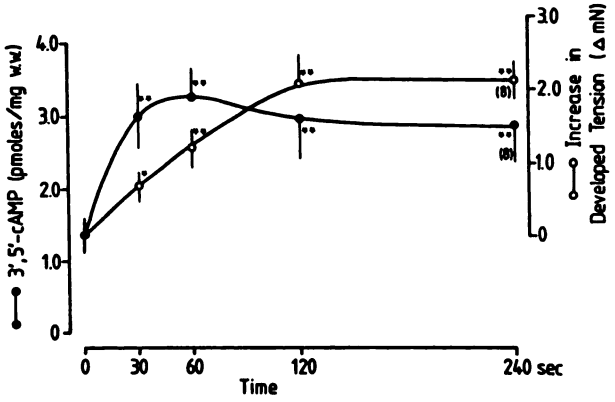

FIG. 3. Time course of the effects of 300 nM isoprenaline on contractile force and cAMP content in the isolated electrically driven muscle strips of human right atrial appendages, obtained from patients without apparent heart failure undergoing elective coronary artery bypass grafting. Left ordinate, cAMP in pmol/mg wet weight; right ordinate, increase in contractile force in mN; abscissa, time after the administration of isoprenaline in s. ● cAMP, ○ contractile force. Each value (except control cAMP content, $n = 16$) is the mean ± SEM (bar) of eight experiments. $^{**}P < 0.01$, $^{*}P < 0.05$ versus the corresponding values before isoprenaline administration. From Ikezono et al. (1987) and used with permission.

In the right atrium, both β_1- and β_2-ARs are involved in the isoprenaline-induced increase in intracellular cAMP, because the increase could be inhibited by the selective β_1-AR antagonist, bisoprolol, and the selective β_2-AR antagonist, ICI 118,551, in the same concentration range (3 to 30 nM). However, ICI 118,551 caused a more pronounced inhibition of the isoprenaline-induced cAMP increase than did bisoprolol (Ikezono et al., 1987), supporting the idea (see above) that in the human heart β_2-ARs seem to be more effectively coupled to AC than are β_1-ARs.

It should be noted, however, that some controversies exist as to whether the increase in intracellular cAMP accounts for all β-AR-mediated effects in the heart. It has recently been shown that β-AR agonists through activation of the G_s protein can directly activate cardiac L-type calcium channels. This process does not require the formation of cAMP (for references, see Birnbaumer, 1990). Whether such an effect occurs in the human heart is not known at present.

C. Functional Importance

As mentioned, the first evidence for the coexistence of β_1- and β_2-ARs in the human heart came from functional in vitro studies of isolated electrically driven right auricles (Åblad et al., 1974). These showed that the β_1-AR-selective antagonist metoprolol antagonized the positive inotropic effect of noradrenaline much more potently than it did that of adrenaline; propranolol antagonized the effects of both catecholamines to about the same degree. Because adrenaline is a nonselective β_1- and β_2-AR agonist, and noradrenaline is rather β_1-AR selective (Lands et al., 1967a,b), these data suggested that, at least in the human right atrium, the effects of adrenaline are mediated by β_1- *and* β_2-AR stimulation. Subsequently, these studies have been confirmed and extended by the use of selective β_1- and β_2-AR agonists and/or antagonists.

1. In vitro positive inotropic effects. a. ATRIAL TISSUES. In isolated, electrically driven right atria obtained from patients without apparent heart failure undergoing elective coronary artery bypass grafting, it has consistently been found that isoprenaline and adrenaline cause their positive inotropic effects through stimulation of both β_1- and β_2-ARs. It has been shown with the selective β_1-AR antagonist CGP 20712 A (300 nM, a concentration that occupies >95% of β_1-ARs but <2% of β_2-ARs) and the selective β_2-AR antagonist ICI 118,551 (30 nM, a concentration that occupies >90% of β_2-ARs but <10% of β_1-ARs) that both drugs caused a shift to the right of the concentration-response curves to isoprenaline and adrenaline to about the same degree (Brodde et al., 1989c; Motomura et al., 1990b); this shift was, however, less than could be predicted assuming that only one β-AR subtype was involved in the action of isoprenaline or adrenaline (fig. 4). In addition, the combined use of both antagonists caused a further marked rightward shift of the concentration-response curve to adrenaline and isoprenaline. Similarly, studies in which the β-AR antagonists (−)-propranolol (Gille et al., 1985) and (−)-atenolol (Lemoine et al., 1988) were used showed that both β_1- and β_2-ARs are involved in the positive inotropic action of adrenaline to about the same degree. In addition, bisoprolol (1 to 100 nM) and ICI 118,551 (1 to 100 nM) antagonized the positive inotropic effect of isoprenaline in a concentration-dependent manner (Zerkowski et al., 1986); Schild plots (Arunlakshana and Schild, 1959) with a slope of approximately 0.5 were obtained for both antagonists, an observation that strongly suggests that isoprenaline does not interact with a homogeneous population of β-ARs in the human right atrium.

On the other hand, independently of the antagonist used (propranolol, bisoprolol, atenolol, practolol, CGP 20712 A, or ICI 118,551), several groups have consistently found that in the human right atrium the positive inotropic effect of noradrenaline is mediated predominantly via β_1-ARs; only at very high (nonphysiological) concentrations is a small β_2-AR component detected (Wilson and Lincoln, 1984; Gille et al., 1985; Brodde, 1986; Lemoine et al., 1988; Kaumann et al., 1989a; Motomura et al., 1990b). Thus, linear Schild plots with a slope not significantly different from unity were obtained for bisoprolol (Brodde, 1986), atenolol (Lemoine et al., 1988), practolol (Wilson and Lincoln, 1984), and ICI 118,551 (Wilson and Lincoln, 1984; Brodde et al., 1989a). Moreover, the shift to the right of the concentration-response curve for noradrenaline by CGP 20712 A (300 nM) was only slightly, if at all, enhanced by the addition of ICI 118,551, and the β_2-AR-selective antagonist itself did not cause a significant shift of the noradrenaline concentra-

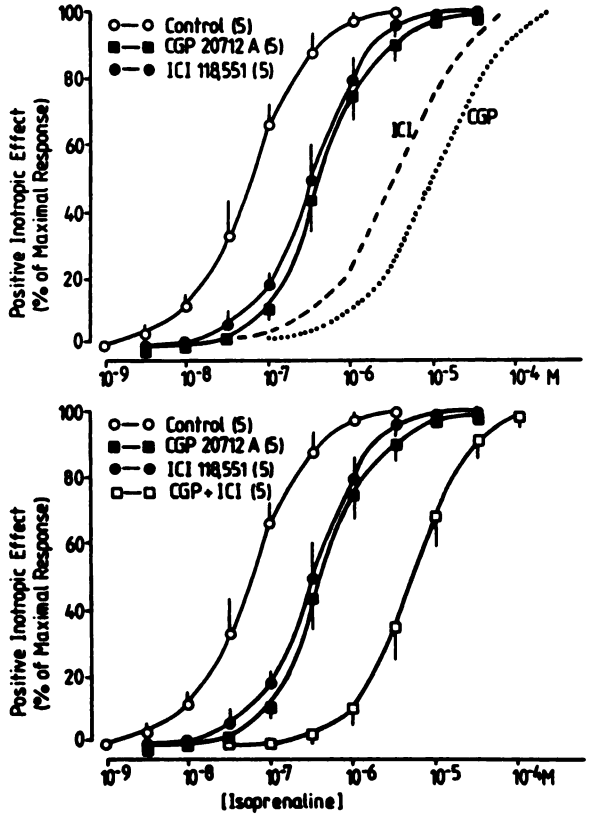

FIG. 4. Antagonism of isoprenaline-induced positive inotropic effect on isolated electrically driven human right atria, obtained from patients without apparent heart failure undergoing elective coronary artery bypass grafting, by 30 nM ICI 118,551 or 300 nM CGP 20712 A, given either alone or in combination. ----- and ·····, respectively (top) indicate theoretical rightward shifts of the concentration-response curve to isoprenaline, assuming that only β_1-ARs (i.e., CGP 20712 A sensitive) or β_2-ARs (i.e., ICI 118, 551 sensitive) are involved in the action of isoprenaline. Bars, SEM. Modified from Motomura et al. (1990b) and used with permission.

tion-response curve (Kaumann et al., 1989a; Motomura et al., 1990b).

Further evidence for the involvement of β_2-ARs in positive inotropic effects of β-AR agonists in the right atrium came from studies in which were used selective β_2-AR agonists such as fenoterol, which in this preparation is a full agonist causing maximal inotropic effects not significantly different from those of isoprenaline (Wilson and Lincoln, 1984). This effect seems to be mediated solely via β_2-AR stimulation as demonstrated by Schild plots with slopes not significantly different from unity for practolol and ICI 118,551. Similarly, in human right atrial appendages, the β_2-AR-selective agonist salbutamol increased contractile force via activation of β_2-ARs only (Ask et al., 1985; Hall et al., 1990); its potency (pEC_{50} values between 5.4 and 5.76) was well in its range for β_2-AR-mediated effects. A role for β_2-ARs is further supported by the finding that 50 nM ICI 118,551, but not 300 nM CGP 20712 A, antagonized the salbutamol effects (Hall et al., 1990). However, the intrinsic activity of salbutamol was low, varying between 39% (Hall et al., 1990) and 59% (Ask et al., 1985) of that of isoprenaline.

Similarly, the β_2-AR-selective agonist procaterol (Yabuuchi, 1977) caused maximal inotropic effects that amounted to approximately 75 to 80% of that of isoprenaline (Zerkowski et al., 1986). Slopes of Schild plots for the antagonism by bisoprolol and ICI 118,551 (Brodde, 1986; Brodde et al., 1989a) were not different from unity, indicating solely β_2-AR stimulation. This has been confirmed in experiments showing that 70 nM ICI 118,551 caused a marked parallel shift to the right of the concentration-response curve for procaterol; the pK_B value for ICI 118,551 was 9.06, which is well within the range of its affinity for β_2-ARs (Buxton et al., 1987).

No data are presently available regarding the functional response of left atrial β_1- and β_2-ARs to β-AR stimulation in *nonfailing hearts*. The only studies published so far have been performed on left atria from patients with MVD and moderate heart failure (NYHA class III to III to IV) (Brodde et al., 1989c, Motomura et al., 1990b). In these studies the positive inotropic effect of isoprenaline and adrenaline was antagonized by CGP 20712 A (300 nM) and ICI 118,551 (30 nM) to about the same degree; combined administration of both antagonists caused a further marked rightward shift of the concentration-response curves. This indicates that, as in the right atrium, both β_1- and β_2-AR stimulation can cause maximal positive inotropic effects. On the other hand, in left atria too, the positive inotropic effect of noradrenaline was only antagonized by 300 nM CGP 20712 A but not by 30 nM ICI 118,551 (Motomura et al., 1990b).

b. VENTRICULAR TISSUES. Studies published to date regarding left ventricular papillary muscles obtained from patients with MVD or patients with HOCM have shown a 300 nM CGP 20712 A-insensitive component in the lower part of the concentration-response curves to isoprenaline and adrenaline (fig. 5). This consisted of up to 60% of the maximal effects of isoprenaline and adrenaline and could be abolished by the addition of 30 or 50 nM ICI 118,551, demonstrating the involvement of β_2-ARs in the positive inotropic actions of isoprenaline and adrenaline (fig. 5; Kaumann and Lemoine, 1987; Kaumann et al., 1989a; Brodde et al., 1989c; Motomura et al., 1990b). Similar data were obtained with (−)-atenolol (Lemoine et al., 1988). However, in contrast to the right and left atria (see above), in left ventricular tissues the β_2-AR-mediated maximal inotropic effect was only 50 to 60% of that to β_1-AR stimulation.

On the other hand, the positive inotropic effect of noradrenaline is mediated as in atria predominantly by β_1-AR stimulation, because it is largely insensitive to ICI 118,551 (fig. 5). A small β_2-AR-mediated component of the positive inotropic action of noradrenaline, however, might be present at high (nonphysiological) concentra-

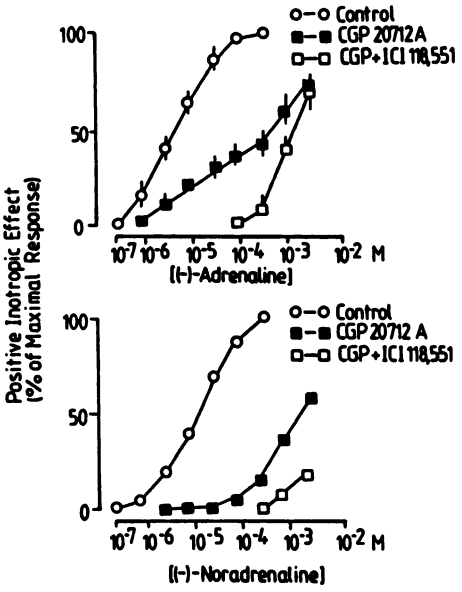

FIG. 5. Antagonism of adrenaline-induced (top) or noradrenaline-induced (bottom) positive inotropic effects on isolated, electrically driven human left papillary muscles, obtained from three patients with HOCM, by 300 nM CGP 20712 A (i.e., blockade of β_1-ARs) or by CGP *plus* 50 nM ICI 118,551. Note that a considerable amount of the positive inotropic effect of adrenaline is insensitive to CGP 20712 A, i.e., is mediated by β_2-ARs, whereas noradrenaline induces β_2-AR-mediated positive inotropic effects only at very high (>10 μM) concentrations. Bars, ±SEM. From Kaumann and Lemoine (1987) and used with permission.

tions (fig. 5; Kaumann and Lemoine, 1987; Lemoine et al., 1988; Kaumann et al., 1989a; Motomura et al., 1990b).

Further support for a β_2-AR contribution to the positive inotropic effects seen in human left papillary muscles came from studies showing that the selective β_2-AR agonist fenoterol produced positive inotropic effects that were insensitive to antagonism by 2 μM atenolol but could be antagonized by 1 μM ICI 118,551 (Mügge et al., 1985). Surprisingly, however, in this study fenoterol caused the same maximal inotropic effect as isoprenaline. In addition, salbutamol was nearly ineffective, although it was an effective (partial) agonist in right atria (see above).

In right ventricular trabeculae from nonfailing hearts, the positive inotropic effect produced by the selective partial β_2-AR agonist zinterol was only approximately 40% of the response resulting from isoprenaline administration (Bristow et al., 1986; Bristow, 1988). This is mediated solely via β_2-AR stimulation being resistant to 0.1 μM of the selective β_1-AR antagonist betaxolol but significantly shifted to the right by 70 nM ICI 118,551 (pK_B 9.3, which is well within the range of its affinity for β_2-ARs).

Thus, taken together, these data strongly support the view that on right and left ventricular tissues of the human heart β_2-ARs can mediate a positive inotropic effect, but these effects are submaximal when compared with β_1-AR-mediated effects.

2. In vivo positive chronotropic effects. The biochemical and in vitro physiological data discussed above show without any doubt that in the human heart both functional β_1- and β_2-ARs coexist. The question arises of whether both β-AR subtypes contribute in vivo to positive inotropic and/or chronotropic effects to β-AR agonists.

Two methods are traditionally used to assess cardiac β-AR function in humans: exercise- and isoprenaline-induced tachycardia (for a recent review, see McDevitt, 1989). In normal volunteers heart rate is controlled by both the sympathetic and the parasympathetic nervous systems (Robinson et al., 1966). At rest the parasympathetic nervous system predominates, but during exercise, tachycardia is caused by a combination of stimulation of sympathetic activity and parasympathetic withdrawal (Robinson et al., 1953). Dynamic exercise is associated with a marked increase in plasma noradrenaline levels (Christensen and Galbo, 1983; Stratton et al., 1983), presumably via release of neuronal noradrenaline (Robertson et al., 1979); thus, exercise-induced tachycardia may be considered as a noradrenaline-induced tachycardia. On the other hand, isoprenaline-induced tachycardia is caused by activation of both β_1- and β_2-ARs. In support of this is the fact that has been known for a long time that selective β_1-AR antagonists inhibit isoprenaline-induced tachycardia much less effectively than do nonselective β-AR antagonists (e.g., propranolol) when both are given in doses that attenuate exercise-induced tachycardia to about the same degree (Brick et al., 1968; Johnsson et al., 1975; Conway et al., 1976; De Plaen et al., 1976; Gugler et al., 1980; Brown et al., 1983, 1986a).

Several studies have shown that ICI 118,551 antagonized isoprenaline-induced tachycardia in doses that had no influence on exercise-induced tachycardia (Tattersfield and Cragg, 1983; Arnold et al., 1985; Vincent et al., 1987; Brodde et al., 1988; Motomura et al., 1990b). Moreover, comparison of the effects of a single dose of ICI 118,551 (25 mg), atenolol (50 mg), and propranolol (80 mg) on exercise- and isoprenaline infusion-induced tachycardia in healthy volunteers showed that 25 mg of ICI 118,551 was equipotent to 50 mg of atenolol in antagonizing isoprenaline-induced tachycardia, whereas 80 mg of propranolol was much more effective (Pringle et al., 1988). Interestingly, a combination of 25 mg ICI 118,551 *plus* 50 mg atenolol caused a shift to the right of the isoprenaline dose-response curve that was now comparable with that produced by propranolol (fig. 6). On the other hand, ICI 118,551 caused only marginal reductions in exercise-induced tachycardia and did not enhance the antagonistic effect of atenolol (fig. 6).

Taken together these data clearly indicate that exercise-induced tachycardia is predominantly, if not exclusively, mediated by β_1-AR stimulation, and isoprenaline-induced tachycardia is mediated by both β_1- and β_2-AR activation to about the same degree. This conclusion has been recently confirmed in the elegant work of Wellstein

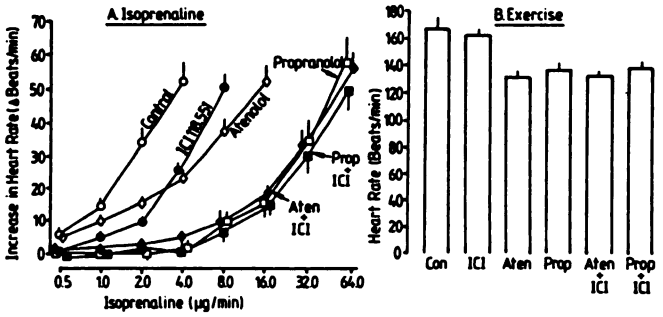

FIG. 6. Effects of atenolol (50 mg, p.o.), ICI 118,551 (25 mg, p.o.), and propranolol (80 mg, p.o.), alone or in combination, on isoprenaline infusion-induced (A) or exercise-induced (B) tachycardia in 6 healthy male volunteers. For further details see text. Bars, SEM. From Pringle et al. (1988) and used with permission.

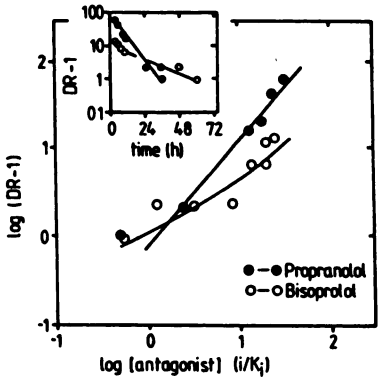

FIG. 7. Schild plot analysis of the in vivo antagonism of isoprenaline infusion-induced increases in heart rate by propranolol (240 mg, orally) or bisoprolol (100 mg, orally) in 18 healthy male volunteers. At certain times after the administration of the β-AR antagonists, dose-response curves for isoprenaline-induced increases in heart rate were determined; simultaneously, plasma concentrations of propranolol and bisoprolol were assessed. Ordinate, dose ratio minus 1 (DR-1) for isoprenaline at different times (see inset); abscissa, antagonist concentration in plasma (i/K_i) at the respective times; i, concentration of antagonist; K_i, its dissociation constant, determined by radioligand-binding studies. From Wellstein et al. (1988) and used with permission.

et al. (1988). Utilizing classical Schild plot analysis of the in vivo antagonism of propranolol and bisoprolol against isoprenaline-induced tachycardia in healthy volunteers, these authors could show that the antagonism of the nonselective β-AR antagonist propranolol resulted in Schild plots with slopes not significantly different from unity, whereas the slope of the Schild plot for the β_1-AR-selective antagonist bisoprolol was 0.68. (fig. 7). It can be calculated that the isoprenaline-induced tachycardia is equally mediated by β_1- and β_2-ARs. In contrast, antagonism by propranolol and bisoprolol against exercise-induced tachycardia had a slope of the Schild plot of unity, confirming that it is mediated nearly exclusively via β_1-ARs.

Further evidence for the involvement of β_2-ARs in the chronotropic response to β-AR agonists came from studies in which the rather β_2-AR-selective agonist terbutaline was used in healthy volunteers. The positive chronotropic effect of terbutaline infusions (0.2 µg/kg/min) was not at all affected by 50 mg of atenolol, a dose that effectively inhibits β_1-AR-mediated effects in humans (Strauss et al., 1986; Levine and Leenen, 1989).

Three possible mechanisms may explain the β_2-AR-mediated increases in heart rate following isoprenaline or terbutaline infusion: β_2-AR-mediated vasodilation causing baroreceptor-mediated reflex vagal withdrawal, activation of presynaptic β_2-ARs resulting in enhanced release of noradrenaline, and direct activation of atrial β_2-ARs. Vagal withdrawal does not considerably contribute to the effects of isoprenaline or terbutaline *infusions* because it has been observed in volunteers after atropine pretreatment that during isoprenaline infusion vagal tone is actually increased, thus blunting the effects of the β-AR agonists (Arnold and McDevitt, 1984, 1986; Arnold et al., 1985; Levine and Leenen, 1989). In addition, simultaneous infusion of angiotensin II to prevent the decrease in diastolic blood pressure induced by isoprenaline did not affect its chronotropic action (McGibney et al., 1983). Thus, because an isoprenaline or terbutaline infusion actually increases vagal tone, the overall contribution of the parasympathetic nervous system to the chronotropic effects of these drugs cannot be excluded, although these effects appear to be rather small.

Additionally, an activation of presynaptic β_2-ARs [whose stimulation leads to enhanced noradrenaline release (Vincent et al., 1982, 1984, 1987; Goldstein et al., 1986; Brodde et al., 1988; Motomura et al., 1990b), resulting in a β_1-AR-mediated tachycardia, and which appear to be present also in the human right atrium (Hill et al., 1987, 1988)] cannot be completely ruled out. In fact, at a higher infusion rate (0.4 µg/kg/min) of terbutaline, atenolol can significantly reduce the chronotropic effect (Levine and Leenen, 1989).

The most striking evidence that direct activation of atrial β_2-ARs causes chronotropic effects in humans comes from a recent study by Hall et al. (1989). These authors showed that, in patients with chronic stable angina, injection of increasing doses of salbutamol into the right coronary artery causes a dose-dependent sinus tachycardia, whereas injections of the same doses of salbutamol into the ascending aorta did not change heart rate, thus ruling out general systemic effects. Tachycardia induced by the intracoronary injection of salbutamol was not affected by i.v. administration of the selective β_1-AR antagonist practolol (8 mg) but was markedly attenuated by i.v. administration of propranolol (4 mg) given 15 min before the injection of salbutamol (fig. 8). In these doses practolol and propranolol equieffectively antagonize exercise-induced tachycardia (Brick et al., 1968) and should cause equal β_1-AR antagonism. Hence, the lack of effect of practolol rules out the possibility that salbutamol may have activated presynaptic β_2-ARs, thereby resulting in an enhanced noradrenaline release and subsequent β_1-AR stimulation (see above). These data strongly support the view that the salbuta-

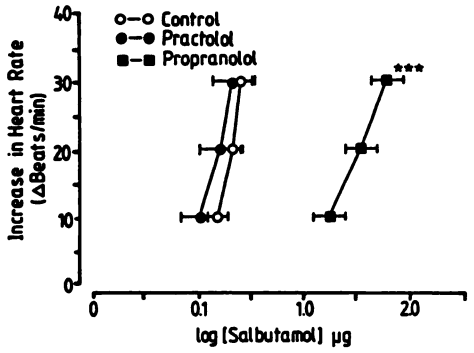

FIG. 8. Effects of practolol (8 mg, i.v., 15 min before experiments) or propranolol (4 mg, i.v., 15 min before experiments) on salbutamol-induced (injected into the right coronary artery) tachycardia in three groups of six patients with chronic stable angina. Bars, ±SEM. ***, mean doses of salbutamol to increase heart rate by 30 beats/min for propranolol and practolol are significantly different ($P < 0.001$). From Hall et al. (1989) and used with permission.

mol-induced tachycardia is due to a direct effect on cardiac β_2-ARs.

3. In vivo positive inotropic effects. Few studies have been performed in which the involvement of β_2-ARs in the positive inotropic effects produced by β-AR agonists was examined because of the difficulty of measuring noninvasively inotropic effects in humans. One noninvasive parameter for quantifying inotropic responses in humans is the determination of the shortening of the electromechanical systole which is rate and afterload independent (Lewis et al., 1977; Belz et al., 1978, 1981, 1985; Stern et al., 1984). In healthy volunteers the shortening of the electromechanical systole (as a measure of positive inotropism) induced by an isoprenaline infusion appears to be mediated by β_1-ARs, because it was antagonized by propranolol and bisoprolol in equal amounts (Wellstein et al., 1988). In contrast, the isoprenaline-induced increase in heart rate was much more effectively antagonized by propranolol than by bisoprolol (see above).

Echocardiographic assessment of the P/V ratio used as a noninvasive measure of inotropy unaffected by changes in preload or afterload (Borow et al., 1982) showed that infusion of graded doses of adrenaline in healthy volunteers dose-dependently increased P/V ratio (Leenen et al., 1988). After pretreatment of the volunteers with propranolol, this increase was completely abolished throughout the whole dose range. In contrast, after pretreatment with atenolol, the highest dose of adrenaline still caused an increase in the P/V ratio. From these data it was concluded that in the left ventricle adrenaline produces its positive inotropic effect predominantly through β_1-AR stimulation, but a small β_2-AR component does seem to be involved, especially at higher adrenaline concentrations. On the other hand, the chronotropic effects induced by adrenaline were only marginally affected by atenolol but were abolished by propranolol, indicating that atenolol was acting predominantly, if not exclusively, at β_1-ARs.

Similar methods for the measurement of positive inotropic effects in healthy volunteers were used to demonstrate that infusion of terbutaline causes positive inotropic effects (Strauss et al., 1986). Pretreatment of the volunteers with a β_1-AR-selective dose of atenolol (50 mg, i.e., a dose that did not affect the β_2-AR-mediated decrease in plasma potassium levels) only moderately attenuated these terbutaline effects, indicating that the positive inotropic effect of terbutaline was, at least partly, mediated by β_2-AR activation. In a later study (Levine and Leenen, 1989) it was shown that pretreatment of the volunteers with atropine potentiated the effects of terbutaline (0.2 μg/kg/min) on increases in P/V ratio (fig. 9). This finding indicated that vagal withdrawal following terbutaline infusion was not the cause of its positive inotropic effects, because vagal tone actually increased, thereby blunting the effects of terbutaline (see above). Because atenolol did not affect the terbutaline effects, an indirect effect of terbutaline via the presynaptic β_2-AR mechanism can be excluded.

The above data seem to indicate that in humans ventricular β_2-AR stimulation might cause some positive inotropic effects in vivo but probably to a lesser extent than does β_1-AR stimulation. This conclusion is in good agreement with the in vitro data obtained from isolated electrically driven human ventricular preparations, in which only β_1-AR stimulation caused maximal positive inotropic effects; β_2-AR stimulation only resulted in submaximal positive inotropic effects (see section II.C.1.b).

D. Positive Inotropic Drugs Acting at Human Cardiac β_1- and/or β_2-Adrenoceptors

Few studies have been performed to characterize the β-AR subtype(s) involved in the effects of positive ino-

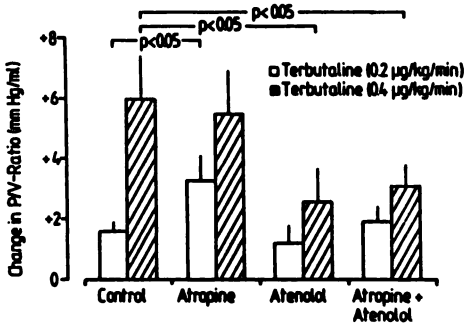

FIG. 9. Increases in P/V ratio (as a measure of inotropy) from baseline (pre-terbutaline levels) in response to terbutaline infusion with and without pretreatment with atenolol (50 mg, p.o., 120 or 150 min before experiments) and/or atropine (0.02 and 0.01 mg/kg, i.v., 30 min and immediately before experiments) in six healthy male volunteers. Note that in the absence of atropine atenolol did not significantly affect the positive inotropic effect of the lower dose of terbutaline but reduced that evoked by the higher dose; after pretreatment with atropine, there was a potentiation of the positive inotropic effect to the lower dose of terbutaline, and after atropine *plus* atenolol the positive inotropic effect of terbutaline was similarly reduced as after atenolol alone (i.e., atenolol antagonized the potentiating effect of atropine). Bars, SEM. From Levine and Leenen (1989) and used with permission.

tropic agents for possible use in the treatment of heart failure.

1. Dopamine. Using isolated electrically driven left papillary muscles of patients with MVD and moderate heart failure (NYHA class III to IV), Brown et al. (1985a) showed that the maximal positive inotropic effect caused by dopamine was approximately 50% of that produced by Ca^{2+}. This effect was antagonized by 1 μM practolol [pK_B value 6.7, which is in the (upper) range of its affinity at β_1-ARs] and by 1 μM ICI 118,551 [pK_B value 7.42, which is closer to its affinity at β_1-AR (6.5 to 7.0) rather than β_2-AR (9.0 to 9.5]. Furthermore, the positive inotropic effect of dopamine was completely abolished in the presence of neuronal uptake inhibition caused by 30 μM cocaine.

The above data indicate that the effects of dopamine are mediated mainly by β_1-AR activation probably indirectly through dopamine-induced release of endogenous noradrenaline. Similarly, in isolated electrically driven left ventricles from patients with either MVD or HOCM and in right ventricles from patients with TF, the *direct* positive inotropic effect of dopamine (indirect effects eliminated by the presence of 5 μM phenoxybenzamine in the organ bath) was approximately 50% of that of isoprenaline (Kaumann et al., 1989b). Unfortunately, in this study no attempt was made to subclassify the β-AR subtype involved in the effects of dopamine.

In isolated right ventricular trabeculae from nonfailing hearts the maximal positive inotropic effect of dopamine was approximately 60% of that of isoprenaline (Port et al., 1990a). Interestingly, in the same tissue taken from transplanted hearts obtained from individuals with normal cardiac function undergoing retransplantation because of graft atherosclerosis, the dopamine effect, but not the isoprenaline effect, was markedly reduced (Port et al., 1990a). Because the transplanted heart is a denervated heart with very low tissue noradrenaline levels (see section V), these data indicate that in the human right ventricle the dopamine effect is predominantly due to an indirect effect via the release of endogenous noradrenaline.

The direct effect of dopamine (in the presence of 5 μM phenoxybenzamine) in isolated electrically driven right atria from patients without apparent heart failure undergoing elective coronary artery bypass grafting was approximately 60 to 70% of that of isoprenaline, whereas in the absence of phenoxybenzamine dopamine caused the same maximal inotropic effect as isoprenaline (Deighton et al., 1990b). In this preparation dopamine was a selective β_1-AR agonist, because its positive inotropic effect was insensitive to 30 nM ICI 118,551, but was markedly antagonized by 300 nM CGP 20712 A. Taken together, these data seem to indicate that in the human heart the positive inotropic effect of dopamine is a mixed direct and indirect (via the release of noradrenaline) β_1-AR-mediated effect.

2. N-methyl-dopamine (epinine). Epinine is the active metabolite of ibopamine, a compound that has been recently introduced for treatment of patients with chronic heart failure (for references, see Henwood and Todd, 1989; Taylor, 1989). On isolated electrically driven right atria, epinine caused the same maximal positive inotropic effect as isoprenaline (Deighton et al., 1990b). This action appeared to be mediated by both β_1- and β_2-ARs, because it was antagonized by 30 nM ICI 118,551 and 300 nM CGP 20712 A to about the same degree; furthermore, a combination of both antagonists caused a further rightward shift of the concentration-response curve of epinine. An indirect component of epinine's action could be excluded because addition of phenoxybenzamine to the bathing medium had no influence on the maximal positive inotropic effect. Thus, in human right atria epinine behaves like adrenaline, i.e., activating both β_1- and β_2-ARs with nearly the same affinity. It should be noted, however, that the potency of epinine at cardiac β-ARs is approximately 10 to 50 times less than are its actions at peripheral dopamine-1 and dopamine-2 receptors (Brodde, 1990; Semeraro et al., 1990). Thus, it is uncertain whether epinine, used in the treatment of chronic heart failure, reaches plasma concentrations sufficient to activate cardiac β-ARs.

3. Dobutamine. On isolated right ventricular trabeculae and left ventricular papillary muscles from nonfailing hearts, dobutamine evokes maximal positive inotropic effects similar to those caused by isoprenaline (Bristow, 1988; Böhm et al., 1988a; Näbauer et al., 1988). Thus, dobutamine is a full agonist on human left ventricular preparations; whether these effects are mediated by β_1- or β_2-AR or both β-AR subtypes has not been investigated.

On isolated electrically driven right atria from patients undergoing elective coronary artery bypass grafting, dobutamine produces its positive inotropic effect via activation of both β_1- and β_2-ARs, because the β_1-AR antagonist bisoprolol (1 to 100 nM) and the β_2-AR antagonist ICI 118,551 (1 to 100 nM) antagonized the positive inotropic effect of dobutamine in the same concentration range. Furthermore, for both antagonists Schild plots with slopes significantly <1.0 were obtained (Zerkowski et al., 1986). However, 100 nM bisoprolol caused a greater rightward shift of the concentration-response curve of dobutamine than did 100 nM ICI 118,551, indicating that dobutamine might have some β_1-AR selectivity. Similarly, 300 nM CGP 20712 A caused a larger rightward shift of the concentration-response curve to dobutamine than did 30 nM ICI 118,551; the latter compound did, however, cause a significant shift to the right. Moreover, addition of ICI 118,551 to CGP 20712 A produced a further significant rightward shift (Bals et al., 1991), indicating that in human right atria both β_1- (to a greater extent) and β_2-ARs (to a minor extent) are involved in the positive inotropic action of dobutamine.

4. Prenalterol. Prenalterol is a β_1-AR partial agonist (Kenakin and Beek, 1980, 1982; Hedberg et al., 1980; Cook et al., 1984; Kenakin, 1985; Gurden et al., 1989). On isolated electrically driven right ventricular strips of patients with TF as well as on right atria from patients undergoing elective coronary artery bypass grafting, prenalterol was found to be completely ineffective or very weak in causing positive inotropic effects (Wilson and Lincoln, 1984; Ask et al., 1985). Moreover, prenalterol did not activate right atrial AC but did competitively inhibited isoprenaline-stimulated AC activity (Brodde et al., 1984).

5. Xamoterol. Xamoterol is a selective β_1-AR partial agonist (Nuttall and Snow, 1982; Cook et al., 1984; Malta et al., 1985) having, in guinea pig papillary muscles (Hattori et al., 1987) and kitten atria and papillary muscles, an intrinsic activity of approximately 30 to 60% of that of isoprenaline (Gurden et al., 1989; Lemoine et al., 1989a). These effects are mediated solely via β_1-ARs. However, in the failing as well as nonfailing human heart it failed to increase contractile force in either the right atria (Böhm et al., 1990c; Bals et al., 1991) or in the left papillary muscles (Lemoine et al., 1989a; Böhm et al., 1990c); weak positive inotropic effects, mediated selectively by β_1-AR stimulation, could be only obtained in the presence of forskolin or the PDE inhibitor milrinone (Böhm et al., 1990c). On the other hand, in both tissues xamoterol behaved as an antagonist against 1 μM noradrenaline-induced positive inotropic effects (Böhm et al., 1990c). Similarly, it only marginally and inconsistently activated AC in human ventricular myocardium (Lemoine et al., 1989a).

6. Denopamine (TA-064). Denopamine is a β_1-AR partial agonist (Bing et al., 1984; Naito et al., 1985; Yokoyama et al., 1988; Lemoine et al., 1989b) that has been used in the long-term treatment of patients with heart failure (Kino et al., 1983; Thormann et al., 1985). Bristow et al. (1986) showed that on isolated electrically driven right ventricular trabeculae from nonfailing hearts denopamine increased contractile force, the maximal effect being approximately 50% of that of isoprenaline. In isolated electrically driven human right atria, the maximal positive inotropic effects of denopamine were approximately 75 to 80% of those produced by isoprenaline; this action could be antagonized by 10 nM CGP 20712 A but not by 30 nM ICI 118,551, thus confirming its β_1-AR selectivity (Bals et al., 1991). However, denopamine only marginally activates AC activity in human right atrial (O.-E. Brodde, unpublished data) and left ventricular membranes (Bristow et al., 1989); this might be due to its partial agonist activity and/or the fact that β_1-ARs in the human heart are not very efficiently coupled to AC.

7. RO 363. RO 363 is a selective β_1-AR partial agonist with an intrinsic activity similar to that of isoprenaline (Iakovidis et al., 1980; McPherson et al., 1984). In isolated electrically driven right atria of patients undergoing elective coronary artery bypass grafting, this compound is nearly a full agonist causing maximal increases in contractile force similar to those produced by isoprenaline. This effect was antagonized by 100 nM CGP 20712 A (pK_B value 9.29) but not at all affected by 70 nM ICI 118,551 (Buxton et al., 1987; Summers et al., 1989), indicating that the effect of RO 363 is mediated solely by β_1-AR stimulation.

8. Dopexamine. Dopexamine is an inotropic agent that has approximately a 10-fold greater affinity for β_2- than for β_1-ARs and also is a rather selective dopamine-1 receptor agonist (Brown et al., 1985b,c; Smith et al., 1987; Böhm et al., 1989b; Brodde and Zerkowski, 1989). In addition, it is a potent inhibitor of neuronal uptake of noradrenaline (Bass et al., 1987; Mitchell et al., 1987; Nedergaard, 1989). In isolated electrically driven human right atrial appendages from patients undergoing elective coronary artery bypass grafting, it causes a positive inotropic effect that amounted to approximately 30 to 50% of the maximal effect caused by isoprenaline (Brodde, 1988; Summers et al., 1989; Brodde and Zerkowski, 1989).

Controversies exist at present as to which β-AR is involved in this effect of dopexamine. Thus, it has been shown (Brodde and Zerkowski, 1989) that the concentration-response curve for dopexamine was not affected by 10 nM bisoprolol but was significantly shifted to the right by 10 nM ICI 118,551. In contrast, the β_1-AR antagonist CGP 20712 A (100 nM) has been shown to preferentially shift the concentration-response curve for dopexamine to the right at the bottom, whereas ICI 118,551 (70 nM) preferentially shifted it at the top (Summers et al., 1989). On the other hand, propranolol (1 μM) led to a parallel shift of the dopexamine concentration-response curve to the right (Summers et al., 1989). Thus, in right atria dopexamine predominantly acts via β_2-ARs, although a β_1-AR component (direct or indirect) cannot be excluded.

Similar controversial data were reported for human right and left ventricular preparations. In nonfailing right and left ventricles, the maximal effect of dopexamine is approximately 60% of that of isoprenaline (Böhm et al., 1989b; Port et al., 1990a). However, in ventricular preparations from severely failing hearts due to idiopathic DCM, dopexamine's actions were markedly attenuated (Port et al., 1990a) or nearly not detectable (Böhm et al., 1989b) despite the fact that in these hearts β_2-AR function is (at least partly) preserved (see below).

Interestingly, in the transplanted denervated heart with very low tissue noradrenaline levels, the effect of dopexamine also was markedly attenuated (Port et al., 1990a), indicating that most of the drug's positive inotropic action in human ventricular tissues is indirect, as has also been shown in right atria from rabbits treated with reserpine (Mitchell et al., 1987).

Controversial data have been reported concerning the effects of dopexamine in isolated left papillary muscles from patients with MVD and moderate heart failure

(NYHA class III). The positive inotropic effect of dopexamine (maximum approximately 20% of that of isoprenaline) was antagonized by both the β_2-AR antagonist ICI 118,551 (30 nM at lower dopexamine concentrations) and the β_1-AR antagonist CGP 20712 A (300 nM at higher dopexamine concentrations) (Brodde and Zerkowski, 1989). On the other hand, dopexamine caused positive inotropic effects only in the presence of the PDE inhibitor milrinone; this effect was selectively antagonized by 50 nM ICI 118,551 but not by 300 nM CGP 20712 A (Böhm et al., 1989b).

III. β_1- and β_2-Adrenoceptors in the Failing Human Heart

Heart failure is defined as the inability of the heart to supply peripheral organs with sufficient amounts of blood for their metabolic requirements (Braunwald, 1988). Although various disease processes such as ischemia, abnormal loading conditions, immunological damage, or exposure to toxic substances may lead to heart failure, including both abnormal systolic and diastolic function (Dougherty et al., 1984; Soufer et al., 1985; Given et al., 1985; Blaustein and Gaasch, 1987; Grossman, 1990), the characteristic symptoms of heart failure are caused by a common pathophysiological pathway, i.e., the interplay among the failing myocardium, the resulting antegrade tissue hypoperfusion and retrograde congestion, and the activation of two primary compensatory mechanisms, activation of both the sympathetic nervous system and the renin-angiotensin-aldosterone system (for recent reviews, see Packer, 1988; Cohn, 1990).

Because of the reduced renal perfusion and activation of the renin-angiotensin-aldosterone system as well as the increased release of arginine vasopressin (Goldsmith et al., 1983), the circulating blood volume is expanded. The increase in volume of the cardiac chambers and subsequent stretching of the myocardium enables the recruitment of the Frank-Starling mechanism, thus increasing cardiac output. However, preload recruitment increases cardiac output only within a certain range, and continued increases in residual cardiac blood volume will finally lead to the clinical characteristics of volume overload, i.e., enhanced heart size, increased central venous pressure, and peripheral and/or pulmonary oedema. Increased sympathetic activity [which recently has been directly demonstrated by studies with peroneal nerve recordings of sympathetic nerve traffic in patients with chronic heart failure (Leimbach et al., 1986)] results in a stabilization of central venous pressure through several mechanisms: vascular α-AR-mediated vasoconstriction, increase in the force of contraction (positive inotropic effects), increase in rate of contraction (positive chronotropic effects), and increase in the rate of relaxation (positive lysotropic effects). Thus, augmented sympathetic nervous system activity aids the failing heart to maintain blood pressure and adequate perfusion of vital organs but leads to the well-known clinical symptoms of tachycardia.

Various authors have shown that in patients suffering from chronic heart failure plasma noradrenaline levels are elevated, and this increase seems to be related to the severity of the disease (Chidsey et al., 1962, 1965; Chidsey and Braunwald, 1966; Thomas and Marks, 1978; Francis et al., 1982, 1984; Levine et al., 1982; for additional references, see Francis and Cohn, 1986). In fact, it has been suggested that plasma noradrenaline levels may serve as a predictor of the prognosis of the patients (Cohn et al., 1984; Rector et al., 1987). The mechanism underlying this increase in plasma noradrenaline is unclear at present. It might be due to an increase in noradrenaline spillover from organs exhibiting increased sympathetic drive [heart and kidney (Rose et al., 1983; Swedberg et al., 1984; Hasking et al., 1986) but not lung (Hasking et al., 1986) or skeletal muscle (Rose et al., 1985)] or to a markedly decreased cardiac neuronal uptake of noradrenaline (Petch and Nayler, 1979; Rose et al., 1983, 1985; Sandoval et al., 1989), or to both. Either of these mechanisms might well lead to the paradox often described in severe heart failure, namely, that cardiac noradrenaline stores are depleted (Chidsey et al., 1965; Kawai et al., 1983; Pierpont et al., 1987) whereas plasma noradrenaline levels are markedly elevated. In addition, because the human heart is strongly dependent on neuronal uptake for in vivo removal of circulating noradrenaline (Goldstein et al., 1988), a decreased cardiac neuronal uptake of noradrenaline and/or an enhanced cardiac sympathetic nervous activity may cause sufficient increases in synaptic cleft noradrenaline such that concentrations of the amine that produce pharmacological effects are reached (see below).

It is now well established that after prolonged stimulation of β-ARs with β-AR agonists (exogenously applied or endogenously sustained released) the cellular response to β-AR agonists will be blunted. This "desensitization" process is consistently associated with a decreased number of β-ARs and/or an impaired ability of β-AR agonists to stimulate AC (Harden, 1983; Stiles et al., 1984; Hertel and Perkins, 1984; Lefkowitz and Caron, 1985; Hausdorff et al., 1990). Thus, it could be expected that in heart failure β-AR responsiveness might decrease due to the elevated release of endogenous noradrenaline (at least locally in the heart; see Bristow et al., 1988). Indeed, this seems to be the case: Bristow et al. (1982) were the first to demonstrate that in severe heart failure cardiac β-AR number and the inotropic response to isoprenaline, but not to calcium, were markedly depressed when compared with nonfailing hearts. Subsequently, many authors have confirmed and extended these observations. At present there seems to be no doubt that a common feature of heart failure is a reduced number of cardiac β-ARs which appears to be due to a real loss of tissue content of β-AR and not a redistribution to intracellular sites (Denniss et

al., 1989a; Murphree and Saffitz, 1989). This is accompanied by a decrease in functional responsiveness, and the extent of β-AR desensitization is related to the degree of heart failure independently of whether judged clinically by NYHA class or left ventricular ejection fraction (for references, see Bristow et al., 1985, 1990; Brodde, 1987, 1989; Erdmann, 1988; Jones et al., 1989; Barnett, 1989; Maisel and Michel, 1989; Feldman and Bristow, 1990).

Thus, in general, cardiac β-AR number and contractile responses to β-AR stimulation are more reduced the more advanced the disease is. Controversies, however, still exist as to whether only β_1-AR function or both β_1- and β_2-AR function are affected. In the next section an attempt is made to summarize the diverse findings in this field and to try to relate the differential changes to the different etiologies of heart failure.

A. Idiopathic Dilated Cardiomyopathy

Cardiac tissue obtained from patients undergoing heart transplantation because of end-stage DCM have been intensively investigated. There is general agreement that in right and left ventricular membranes and in right and left atria of these patients the number of β-ARs is markedly reduced (Bristow et al., 1982, 1986, 1991; Brodde et al., 1986c, 1989b; Fowler et al., 1986; Böhm et al., 1988a,b, 1989b, 1990a; Limas et al., 1989b; Denniss et al., 1989a,b; Vago et al., 1989; Steinfath et al., 1991a,b). Moreover, in all studies in which alterations in β_1- and β_2-ARs were determined, a consistent selective loss of β_1-ARs was observed, whereas number of β_2-ARs was not changed (fig. 10; table 3). Thus, the β_1-AR to β_2-AR ratio in the ventricles is shifted from 80:20% (cf. table 2) to approximately 60:40%.

In patients with biventricular failure the β_1-AR-selective reduction can be observed in both right and left ventricles, whereas in patients with isolated right ventricular failure from primary pulmonary hypertension only the number of β_1-ARs is decreased in the right ventricle; total number of β-, β_1-, and β_2-ARs in the left ventricle is not changed and is nearly identical with that determined in nonfailing hearts (Bristow et al., 1986). A nearly identical chamber-specific down-regulation of right ventricular β-ARs, without any changes in left ventricular β-ARs, has been recently observed in an experimental dog model with right heart failure produced by tricuspid avulsion and progressive pulmonary artery constriction (Liang et al., 1989). These data further support the view that local rather than systemic increases in adrenergic drive occur in heart failure (see section III).

The reduction in receptor number is accompanied by a marked reduction in the functional responsiveness of the β-AR to β-AR stimulation. Thus, in all studies, isoprenaline-stimulated AC was reduced in membranes from right and left ventricles (Bristow et al., 1982, 1984, 1989, 1991; Böhm et al., 1989a, 1990a; Denniss et al., 1989a,b; Karliner and Scheinman, 1988). Close inspection of the data reveal, however, that in the majority of studies the reduction in maximal AC activation by isoprenaline was less than the maximal decrease in the number of β_1-ARs. In contrast, the β_1-AR-mediated activation of ventricular AC by the selective β_1-AR partial agonist denopamine was reduced by approximately 75%, i.e., to a similar extent to that of β_1-AR density (Bristow et al., 1989).

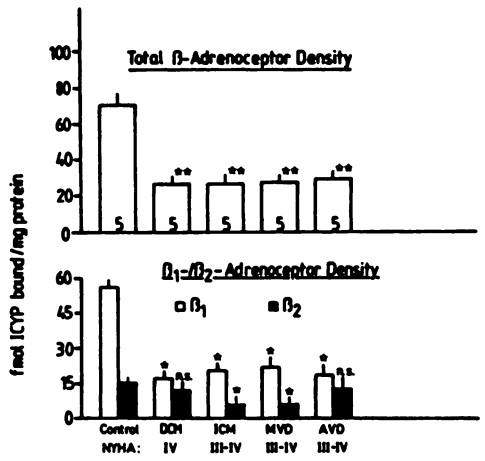

FIG. 10. Left ventricular total β-, β_1-, and β_2-AR changes in different forms of heart failure. Top, Total β-AR density, determined from Scatchard (1949) analysis of ICYP binding in fmol ICYP specifically bound/mg protein; bottom, β_1- and β_2-AR density, determined by inhibition of ICYP binding (50 pM) with a β_1-AR-saturating concentration of the highly selective β_1-AR antagonist CGP 20712 A (300 nM) in fmol ICYP specifically bound/mg protein. Columns, means (bars, SEM) of 5 experiments each. $^{**}P < 0.01$, $^{*}P < 0.05$ versus the corresponding values in controls (i.e., left ventricular total β-, β_1-, and β_2-ARs in nonfailing hearts); n.s., not significantly different from the corresponding value in controls. Modified from Steinfath et al. (1991a,b) and used with permission.

On the other hand, β_2-AR activation of AC in these severely failing hearts by the selective β_2-AR agonist zinterol was only slightly (approximately 30%) reduced (Bristow et al., 1989), indicating that the unchanged number of β_2-ARs in end-stage DCM is accompanied by only a mild "uncoupling" of the receptor from the AC. The preservation of the ability of β_2-ARs to activate AC may be the reason for the less reduced ability of isoprenaline to activate AC because this is achieved mainly by β_2-AR stimulation (cf. fig. 2).

Nonreceptor-mediated activation of the AC by GTP or its nonhydrolyzable analogue Gpp(NH)p is also reduced in patients with end-stage DCM (Feldman et al., 1988; Karliner and Scheinman, 1988; Ransnäs et al., 1988; Denniss et al., 1989a,b; Böhm et al., 1990a; Bristow et al., 1991). This observation indicates that not only β-ARs but also the signal transduction pathway might be affected in severe heart failure. Recent studies suggest that in these patients the amount of G_i protein is increased. Thus, in four studies (Feldman et al., 1988; Neumann et al., 1988; Böhm et al., 1990a; Bristow et al., 1991) in which ventricular membranes of patients with

TABLE 3

Number of ventricular β-AR and subtype distribution in end-stage DCM and end-stage ICM in patients with biventricular failure

Tissue and ligand	B_{max} (fmol/mg protein)	β_1-AR to β_2-AR ratio (%)	References
End-stage DCM			
Left ventricle			
ICYP	43 ± 3 (12)*	60:40	Bristow et al. (1986)
ICYP	51 ± 2 (47)	64:36	Bristow et al. (1991)
[^{3}H]CGP 12177	34 ± 6 (6)	ND†	Böhm et al. (1990a)
[^{3}H]CGP 12177	58 ± 5 (5)	63:37	Böhm et al. (1989b)
ICYP	31 ± 7 (8)	62:38	Brodde et al. (1986c)‡
ICYP	26 ± 3 (5)	60:40	Steinfath et al. (1991a, b)
Right ventricle			
ICYP	40 ± 5 (9)	61:39	Bristow et al. (1986)
ICYP	48 ± 3 (47)	65:35	Bristow et al. (1991)
ICYP	33 ± 7 (8)	64:36	Brodde et al. (1986c)‡
End-stage ICM			
Left ventricle			
ICYP	63 ± 4 (24)	69:31	Bristow et al. (1991)
ICYP	26 ± 3 (5)	78:22	Steinfath et al. (1991a, b)
ICYP	35 ± 6 (7)	77:23	Brodde et al. (1989b)§
[^{3}H]CGP 12177	41 ± 6 (6)	ND	Böhm et al. (1990a)
Right ventricle			
ICYP	66 ± 5 (24)	63:37	Bristow et al. (1991)
ICYP	28 ± 7 (8)	78:22	Brodde et al. (1989b)§

* Mean ± SEM (number of experiments, performed on different hearts).
† Not determined.
‡ Recalculated from Brodde et al. (1986c) and unpublished data.
§ Recalculated from Brodde et al. (1989b) and unpublished data.

end-stage DCM were used, pertussis toxin treatment resulted in increased ADP ribosylation compared to non-failing hearts (table 4). Such an increase in G_i protein may explain the reduced ability of GTP and Gpp(NH)p to activate AC.

It is interesting to note that a similar increase in G_i protein has been recently described in cultured rat heart cells after pretreatment with noradrenaline (Reithmann et al., 1989). Moreover, in rats chronically treated with isoprenaline, ventricular G_i protein (as assessed by pertussis toxin-catalyzed ADP ribosylation) and G_i messenger RNA levels are elevated, and this increase can be prevented by simultaneous propranolol administration (Eschenhagen et al., 1991a,b). Thus, it might be possible

TABLE 4

Ventricular G proteins and AC activation in end-stage DCM

G proteins*		AC activation					References
G_s	G_i	Isoprenaline (100 μM)	Gpp(NH)p (100 μM)	NaF (10 mM)	Forskolin (100 μM)	Mn^{2+} (10 mM)	
↔↑	↑	ND‡	↓	↔	↓	ND	Feldman et al. (1988)
↔	↑	↓§	↓	ND	↔§	ND	Böhm et al. (1990a), Schnabel et al. (1990)
↔	↑	↓	↓	↔	↓	↔	Bristow et al. (1991)
ND	↑	ND	ND	ND	ND	ND	Neumann et al. (1988)
↓‖	ND	ND	↓¶	ND	ND	ND	Ransnäs et al. (1988)
ND	ND	↓	↓	↓	ND	ND	Karliner & Scheinman (1988)
↑**	↑**	ND	ND	ND	ND	ND	Feldman et al. (1989)
↔**	↑**	ND	ND	ND	ND	ND	Eschenhagen et al. (1991c)

* The amount of G_s was assessed by cholera toxin-catalyzed [^{32}P]ADP ribosylation; the amount of G_i was assessed by pertussis toxin-catalyzed [^{32}P]ADP ribosylation. ↔, no change; ↑, increase; ↓, decrease.
† G_s function, assessed by cyc$^-$ reconstitution assay and found to be unaltered also.
‡ Not determined.
§ 10 μM.
‖ Determined with an antibody against $G_{s\alpha}$.
¶ 100 μM GTP.
** mRNA levels for $G_{s\alpha}$ and $G_{i\alpha}$ were determined by Northern blotting.

that the increase in ventricular G_i protein seen in patients with DCM may be due to the chronic exposure of the heart to elevated, released locally in the heart, catecholamines.

Divergent results have been reported concerning the effects of forskolin on AC activation in end-stage DCM. Either no differences in activation of ventricular AC in nonfailing hearts and in end-stage DCM by forskolin (10 μM) (Böhm et al., 1989a, 1990a) or a significantly reduced activation of AC in end-stage DCM by forskolin (100 μM) have been reported (Feldman et al., 1988; Bristow et al., 1991). Furthermore, inactivation of G_i by pertussis toxin treatment abolished the reduced ability of Gpp(NH)p and forskolin to activate AC (Feldman et al., 1988). Similarly, the increase in G_i protein in cultured rat heart cells after pretreatment with noradrenaline (see above) was associated with a diminished maximal activation of AC by GTPγS and forskolin but *not* by NaF (Reithmann et al., 1989). In this model system, too, inactivation of G_i prior to activation of AC by forskolin restored its ability to achieve full activation. On the other hand, manganese-induced activation of AC seems not to be altered in end-stage DCM (Bristow et al., 1991).

Divergent results have been reported concerning changes in G_s protein in DCM (table 4). Using cholera toxin-catalyzed ADP ribosylation, three groups (Feldman et al., 1988; Schnabel et al., 1990; Böhm et al., 1990b; Bristow et al., 1991) found no changes in the amount of ventricular G_s protein, and in a reconstitution assay the functional activity of G_s was found to be similar in patients with end-stage DCM and normal controls (Feldman et al., 1988). In contrast, Ransnäs et al., (1988) using an antibody against $G_{s\alpha}$ found a slight decrease in the amount of ventricular G_s accompanied by a marked decrease in its functional activity as assessed by activation by GTP. In lymphocytes of patients with DCM, G_s (as assessed by cholera toxin-catalyzed ADP ribosylation) was found to be markedly decreased (Horn et al., 1988) or unchanged (Maisel et al., 1990c) when compared with that in healthy controls. In the latter study lymphocyte G_i protein (assessed by pertussis toxin-catalyzed ADP ribosylation) was also not different from that in healthy controls. However, treatment of anticoagulated blood for 4 h at 37°C with pertussis toxin, which completely ADP ribosylated the lymphocyte pertussis substrates, significantly enhanced prostaglandin E_1-produced lymphocyte cAMP accumulation in the patients with DCM but not in the healthy controls (Maisel et al., 1990c). This indicates that in patients with DCM lymphocyte cAMP generation is under a greater tonic inhibitory influence by G_i than in normal subjects.

On the other hand, most investigators (Bristow et al., 1984, 1991; Feldman et al., 1988), with one exception (Karliner and Scheinman, 1988), found no differences in 10 mM NaF-stimulated AC activity in membranes derived from nonfailing hearts and hearts from patients with DCM (table 4). Because NaF in this concentration (10 mM) is believed to activate predominantly G_s protein with little effect on G_i protein (Katada et al., 1984), the lack of any differences in the effects of NaF between nonfailing hearts and hearts from patients with DCM supports the view of an unchanged G_s activity. On the other hand, it is worthwhile noting that in nearly all animal models of heart failure the amount of cardiac G_s protein appeared to be decreased (for references, see Horn and Bilezikian, 1990).

Taken together, although certainly future experiments have to clarify the changes in cardiac G_i and/or G_s proteins in end-stage DCM, there seems to be no doubt that in this disease the *ratio* of G_s to G_i is decreased.

The decreased β-AR density and the diminished responsiveness of the AC activation to β-AR stimulation is accompanied by reduced β-AR inotropic responses of ventricular and atrial preparations derived from patients with end-stage DCM. Various studies have shown a decreased maximal inotropic response of right atria, right ventricular trabeculae, and left ventricular papillary muscles to isoprenaline stimulation (Bristow et al., 1982, 1984, 1986, 1991; Ginsburg et al., 1983; Schmitz et al., 1987, 1989a; Bristow, 1988; Neumann et al., 1988; Böhm et al., 1988b, 1990a), whereas the maximal inotropic effect to Ca^{2+} was only marginally reduced, if at all.

Few studies have investigated whether the selective decrease in β_1-ARs with only a moderate attenuation of β_2-AR responses in DCM also can be demonstrated in physiological experiments. Comparison of the effects of isoprenaline with those of the β_1-AR partial agonist denopamine and the selective β_2-AR agonist zinterol in right ventricular trabeculae from nonfailing hearts and those from patients undergoing heart transplantation because of end-stage DCM showed that in the severely failing hearts the positive inotropic effect of isoprenaline (acting via β_1- and β_2-ARs) was reduced by approximately 60%, whereas that of the partial agonist denopamine (acting solely via β_1-ARs) was nearly abolished (Bristow et al., 1986). The positive inotropic effect of zinterol acting solely via β_2-ARs, however, was only slightly, but not significantly, attenuated (fig. 11). Similarly, the positive inotropic effect of dopamine [acting directly and indirectly at β_1-ARs (see above)] was more reduced than that of isoprenaline (fig. 11; Bristow, 1988; Port et al., 1990a). This can be explained by a selective reduction in β_1-ARs and depletion of cardiac noradrenaline stores. Furthermore, inotropic responses to dobutamine which in the human heart acts mainly through β_1-AR stimulation with a small β_2-AR component (see section II.D.3.) were more reduced than responses to isoprenaline in right and left ventricular tissue from end-stage DCM (fig. 11; Bristow, 1988; Böhm et al., 1989b; Näbauer et al., 1988).

Data obtained using right atrial strips from patients with end-stage DCM showed a similar selective β_1-AR

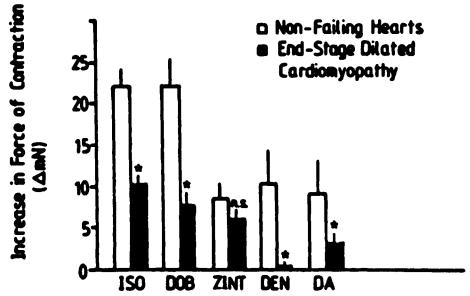

FIG. 11. Maximal positive inotropic effects of β-AR agonists on isolated electrically driven right ventricular trabeculae of nonfailing hearts or hearts derived from patients with end-stage DCM. Ordinate, Maximal increase in contractile force in mN. ISO, isoprenaline; DOB, dobutamine; ZINT, zinterol; DEN, denopamine; DA, dopamine. $^*P <$ 0.05 versus the corresponding values in nonfailing hearts; n.s., not significantly different from the corresponding value in nonfailing hearts. Bars, SEM. From Bristow (1988) and used with permission.

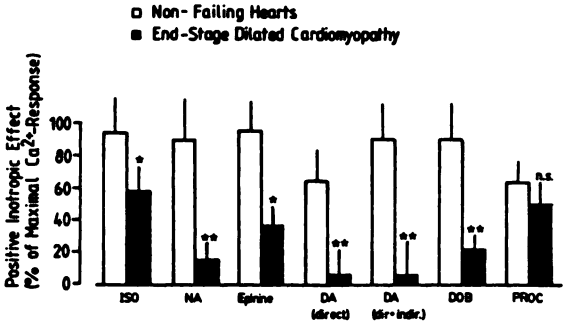

FIG. 12. Maximal positive inotropic effects of β-AR agonists on isolated electrically driven right atria from patients undergoing elective coronary artery bypass grafting without apparent heart failure (nonfailing hearts) and from patients with end-stage DCM. Ordinate, Positive inotropic effect in percentage of maximal Ca^{2+} response (which is not changed in end-stage DCM, see section III.A.). ISO, isoprenaline; NA, noradrenaline; DA (direct), dopamine in the presence of 5 μM phenoxybenzamine (i.e., blockade of neuronal and extraneuronal uptake and α-ARs); DA (dir. + indir.), dopamine in the absence of 5 μM phenoxybenzamine; DOB, dobutamine; PROC, procaterol. Bars, SEM. $^{**}P < 0.01$, $^*P < 0.05$ versus the corresponding values in nonfailing hearts; n.s., not significantly different from the corresponding value in nonfailing hearts. From unpublished data of O.-E. Brodde.

reduction as that observed in right and left ventricles (see Brodde et al., 1986c, 1989b) and displayed less reduction in inotropic effects to nonselective β-AR agonists (isoprenaline and epinine) than to selective β_1-AR agonists (noradrenaline, dopamine, and dobutamine). Inotropic responses to the selective β_2-AR agonist procaterol were only marginally attenuated (fig. 12).

Forskolin-induced maximal inotropic effect in ventricular myocardium derived from patients with end-stage DCM was not different from that obtainable in nonfailing hearts (Bristow et al., 1984; Böhm et al., 1989a), although maximal forskolin-induced activation of AC seems to be diminished, presumably because of the increase in G_i (see above). Similar data were obtained from cultured rat heart cells exposed to high concentrations of noradrenaline which show that G_i protein increases and is accompanied by a reduced forskolin-activated AC. The positive inotropic effect of forskolin, as assessed by increases in contraction velocity of these cells, is, however, sustained (Reithmann and Werdan, 1989). This raises the question of whether the increase in G_i (as determined by pertussis toxin-catalyzed ADP ribosylation) is of functional importance for the contractile response of the failing human heart (Insel and Ransnäs, 1988). It can be argued that for the increase in G_i to be of functional importance one would expect the negative inotropic responses of G_i-coupled receptors present in the human heart such as muscarinic M_2 (Giraldo et al., 1988; Deighton et al., 1990a; Motomura et al., 1990a) or adenosine A_1 receptors (Böhm et al., 1989c) to be enhanced. The data presently available, however, indicate that neither muscarinic M_2 receptor nor adenosine A_1 receptor-mediated negative inotropic effects nor coupling of these receptors to the AC are changed in atrial and ventricular preparations of patients with end-stage DCM (Böhm et al., 1990a; Deighton et al., 1990c). Thus, the functional importance of increases in G_i in end-stage DCM has to be further evaluated.

B. End-Stage Ischemic Cardiomyopathy

Four studies of β-AR changes in patients undergoing heart transplantation because of end-stage ICM have been published (see table 3). A markedly decreased number of β-ARs in right and left ventricular membranes and in right and left atria was reported; the amount of down-regulation appeared to be somewhat less than in end-stage DCM. Divergent results were obtained, however, regarding changes of β_1- and β_2-ARs (table 3). In 24 patients with ICM and end-stage biventricular failure, a selective reduction in the number of β_1-ARs has been reported (Bristow et al., 1991), although this decrease was slightly less than that found in end-stage DCM. In other studies (Brodde et al., 1989b; Steinfath et al., 1991a,b), however, a simultaneous decrease in numbers of β_1- *and* β_2-ARs in patients with ICM was found (fig. 10). The decrease in the number of β-ARs was accompanied by a diminished isoprenaline-induced activation of right atrial and right and left ventricular AC when compared with nonfailing hearts (Brodde et al., 1989b; Böhm et al., 1990a; Bristow et al., 1991). In addition, β_2-AR-mediated activation of AC by zinterol (Bristow et al., 1991) and procaterol (Brodde et al., 1989b) was diminished, findings that support either a decreased number or an uncoupling of the β_2-ARs in end-stage ICM. The decrease in β_2- and/or β_1-ARs in end-stage ICM was accompanied by a reduced inotropic response of right ventricular trabeculae or left ventricular papillary muscles to isoprenaline stimulation when compared with nonfailing hearts. However, again divergent results have been published. One study reported that the decrease in maximal inotropic response to isoprenaline was greater in end-stage ICM than it was in end-stage DCM (Bristow et al., 1991), whereas another study found the opposite (Böhm et al., 1990a), i.e., isoprenaline-induced

increases in contractile force were significantly more attenuated in DCM than in ICM.

Whereas no change in cholera toxin-catalyzed ADP ribosylation of cardiac G_s protein was found (Bristow et al., 1991; Böhm et al., 1990b), results with G_i proteins in end-stage ICM are more controversial (table 5). Using pertussis toxin-catalyzed ADP ribosylation, Böhm et al. (1990a) reported no change in G_i protein and in addition no change in response of left ventricular AC to Gpp(NH)p and forskolin stimulation. Subsequently, however, using an antiserum against $G_{i\alpha}$, the same group found an increase in ventricular G_i protein in end-stage ICM (Böhm et al., 1991). On the other hand, Bristow et al. (1991) showed that in end-stage ICM G_i protein (as assessed by pertussis toxin-catalyzed ADP ribosylation) is increased in a similar way to end-stage DCM and that this was accompanied by decreased Gpp(NH)p and forskolin-stimulated AC.

Clearly, further studies are needed to establish whether or not in end-stage ICM only β_1- or β_1- *and* β_2-ARs are decreased and whether or not G_i protein is increased.

C. Mitral Valve Disease

Many studies of β_1- and β_2-AR changes have been performed in left ventricular tissue from patients undergoing mitral valve replacement due to mitral stenosis or mitral regurgitation. Most laboratories agree that in patients with MVD numbers of β-ARs in left ventricular membranes are decreased compared with nonfailing hearts and that this decrease is likely due to a concomitant decrease in β_1- *and* β_2-ARs (fig. 10; table 6). The β_1-AR to β_2-AR ratio was between 75:25% and 80:20%, which is in the same range as found in left ventricles from nonfailing hearts (table 2).

Determination of β-AR density and subtype distribution by [^{3}H]DHA binding in 12 patients with MVD (NYHA classes I and II) showed a mean β-AR density of 45 fmol/mg protein and a β_1-AR to β_2-AR ratio of 69 (range 65 to 73%) to 31% (range 27 to 35%). (Vago et al., 1984) In a similar study with ICYP binding in 8 patients with MVD (no data concerning ejection fraction or NYHA functional class), a mean number of 30 fmol/mg protein and β_1-AR to β_2-AR ratio of 75:25% was found (Golf et al., 1985a). In the same patients right atrial β-AR density, however, was only slightly lower than that in "normal hearts" (see table 2), being 71 fmol/mg protein with a β_1-AR to β_2-AR ratio of 75:25% (Golf et al., 1985a). In a series of papers, Kaumann and associates (Kaumann et al., 1982, 1989b; Kaumann and Lemoine, 1987; Ferry and Kaumann, 1987) described the number and subtype distribution of β-ARs in left ventricular membranes; in three of these papers (Kaumann et al., 1982, 1989b; Kaumann and Lemoine, 1987) they found, using [^{3}H]bupranolol as a ligand, a B_{max} of 27 to 45 fmol/mg protein and a β_1-AR to β_2-AR ratio of 74:26% (Kaumann and Lemoine, 1987). Nearly the same ratio was obtained by direct binding of [^{3}H]bupranolol to left ventricular β_2-ARs in the presence of a β_1-AR-saturating concentration of the selective β_1-AR antagonist CGP 20712 A (Kaumann and Lemoine, 1987). Surprisingly, the same group found a B_{max} of approximately 66 to 84 fmol/mg protein when using [^{3}H]DHA or ICYP as ligands (Ferry and Kaumann, 1987); however, no data concerning the severity of the disease of these patients with MVD (ejection fraction, cardiac index, or NYHA class) were given.

Recently, Steinfath et al. (1991a,b) showed, using ICYP as a ligand, a markedly reduced number of β-ARs in left ventricles from patients with MVD in NYHA classes III to IV, whereas in patients in NYHA classes II to III the number of left ventricular β-ARs was only slightly less (table 6) than in normal hearts (cf. table 2). Again, β_1-AR to β_2-AR ratio did not change from 80:20% (fig. 10, table 6). Thus, in all of these studies the general finding was a reduced density of β-ARs but an unchanged β_1-AR to β_2-AR ratio when compared with normal hearts.

Baumann et al. (1983) studied numbers of left ventricular β-ARs and AC response to isoprenaline in 16 patients with MVD or combined MVD and AVD and different degrees of heart failure. B_{max} and maximal AC responses gradually decreased with increasing degree of

TABLE 5
Ventricular G proteins and AC activation in end-stage ICM

G proteins*		AC activation					References
G_s	G_i	Isoprenaline (10 μM)	Gpp(NH)p (100 μM)	NaF (10 mM)	Forskolin (10 μM)	Mn^{2+} (10 mM)	
↔	↑	↓†	↓	↔	↓†	↔	Bristow et al. (1991)
↔	↔	↓	↔	ND	↔	ND	Böhm et al. (1990a, b)
ND	↑‡	ND	ND	ND	ND	ND	Böhm et al. (1991)
ND	ND	↓§	ND	ND	↔§	ND	Brodde et al. (1989b)
↔‖	↑‖	ND	ND	ND	ND	ND	Eschenhagen et al. (1991c)

* The amount of G_s was assessed by cholera toxin-catalyzed [^{32}P]ADP ribosylation; the amount of G_i was assessed by pertussis toxin-catalyzed [^{32}P]ADP ribosylation. ↔, no change; ↑, increase; ↓, decrease. ND, not determined.

† 100 μM.

‡ Determined with an antiserum against $G_{i\alpha}$.

§ Determined in right atrial membranes.

‖ mRNA levels of $G_{s\alpha}$ and $G_{i\alpha}$ were determined by Northern blotting.

TABLE 6

Number of myocardial β-AR and subtype distribution in mitral valve disease

Tissue and NYHA class	Ligand	B_{max} (fmol/mg protein)	β_1-AR to β_2-AR ratio (%)	References
Right atrium				
NS*	ICYP	71 ± 6 (8)†	76:24	Golf et al. (1985a)
III	ICYP	63 ± 7 (13)	65:35	Brodde et al. (1989c)
III–IV	ICYP	41 ± 5 (4)	74:26	Brodde et al. (1989c)
IV	ICYP	28 ± 5 (5)	70:30	Brodde et al. (1989c)
Left ventricle				
NS	ICYP	30 ± 3 (8)	75:25	Golf et al. (1985a)
NS	[^{3}H]bupranolol	38 ± 3	ND‡	Kaumann et al. (1982)
NS	[^{3}H]bupranolol	42 ± 5 (3)	74:26	Kaumann and Lemoine (1987)
NS	[^{3}H]DHA	84 ± 11 (5)	ND	Ferry and Kaumann (1987)
NS	ICYP	66 ± 13 (2)	ND	Ferry and Kaumann (1987)
I–II	[^{3}H]DHA	45 ± 2 (6)	69:31	Vago et al. (1984)
II	[^{3}H]bupranolol	27–45	ND	Kaumann et al. (1989b)
II–III	[^{3}H]CGP 12177	18 ± 1 (16)	ND	Böhm et al. (1988a)
II–III	[^{3}H]CGP 12177	60 ± 3 (10)	ND	Schwinger et al. (1990)
II–III	ICYP	62 ± 7 (3)	78:22	Steinfath et al. (1991a, b)
III	ICYP	27 ± 3 (15)	76:24	Brodde et al. (1989c)
III–IV	ICYP	27 ± 3 (5)	80:20	Steinfath et al. (1991a, b)
III–IV	ICYP	20 ± 2 (4)	77:23	Brodde et al. (1989c)
IV	ICYP	14 ± 4 (5)	78:22	Brodde et al. (1989c)
Left atrium				
III	ICYP	56 ± 6 (13)	66:34	Brodde et al. (1989c)
III–IV	ICYP	34 ± 4 (5)	69:31	Brodde et al. (1989c)
IV	ICYP	19 ± 4 (3)	71:29	Brodde et al. (1989c)

* Not specified.
† Mean ± SEM (number of experiments performed in different hearts).
‡ Not determined.

insufficiency at both valves. Moreover, the number of β-ARs and maximal AC stimulation by isoprenaline were significantly inversely related to left ventricular end-diastolic volume and left ventricular end-diastolic pressure, respectively, of the patients. These findings were confirmed and extended (Brodde et al., 1989c). In right and left atria and left papillary muscles from 35 patients with MVD with different degrees of heart failure (NYHA classes III to IV) B_{max} gradually declined when the degree of heart failure increased from NYHA functional class III to class IV (table 6). In patients in NYHA class III atrial β-AR density was only moderately reduced when compared with that in nonfailing hearts (cf. table 2), as previously reported (Golf et al., 1985a). However, left ventricular β-AR density was reduced by >60% (cf. nonfailing hearts, table 2).

The decrease in B_{max} was due to a concomitant decrease in β_1- and β_2-ARs, because the β_1-AR to β_2-AR ratio in the three tissues did not change with the progression of heart failure and was not significantly different from that in nonfailing hearts (table 6).

The decrease in β-AR density was accompanied by a decrease in the contractile response to β-AR stimulation. In isolated electrically driven right atria from patients in NYHA class III and III to IV, the concentration-response curves for the positive inotropic effects of isoprenaline (nonselective), procaterol (β_2-AR selective), and noradrenaline (β_1-AR selective) were shifted to the right by a similar amount when compared with the data obtained in nonfailing hearts; moreover, maximal inotropic effects of all three agonists were decreased to a similar degree. In addition, in isolated electrically driven left papillary muscles maximal responses and the pD_2 values for the positive inotropic effect of isoprenaline gradually declined with the increase in degree of heart failure (Brodde et al., 1989c). Similar results were reported for left papillary muscles of patients with MVD and moderate heart failure (NYHA classes II to III and classes III to IV) in which the maximal positive inotropic effects of isoprenaline, dobutamine, and dopamine (Brown et al., 1986b; Böhm et al., 1989b) were significantly reduced when compared with those in nonfailing hearts.

The forskolin effect on contractile force, however, seems not to be altered in patients with MVD. Thus, when 1 μM forskolin was added to the organ bath at the peak positive inotropic effects to isoprenaline and adrenaline, additional increases in developed tension were produced in both left atria and left papillary muscle which were similar or even greater than the maximal responses to the catecholamines (Brodde et al., 1989c).

Taken together the above data seem to indicate that in MVD there is a gradual decrease of both β-ARs in right and left atria as well as in left ventricles which seems to be related to the severity of the disease (as judged by NYHA functional class or ejection fraction). Moreover, the decrease seems to occur earlier in heart

failure in the left papillary muscle than in right and left atria.

D. Tetralogy of Fallot

Only two studies of β-AR changes in TF have been reported. Kaumann et al. (1989b) found, using [^{3}H]bupranolol as a ligand, a mean number of right ventricular β-ARs between 30 and 48 fmol/mg protein; no β-AR subtypes were assessed in this study. We recently determined, using ICYP binding, β-AR density and subtype distribution in right atria and right ventricles (resected parts of infundibular outflow tract) from 21 children with TF (aged 0.25 to 23 years) undergoing corrective surgery (Brodde et al., 1989b). No relationship between age and right ventricular β-AR density was found; we, therefore, compared right atrial and right ventricular β-AR density in the children with TF and that in nonfailing hearts of adults. The density in right atria (45 ± 5 fmol ICYP bound/mg protein, mean ± SEM; $n = 4$) and right ventricles (34 ± 5 fmol/mg protein, $n = 14$) was significantly lower than in controls (cf. table 2); in contrast, the β_1-AR to β_2-AR ratio in atria and ventricles was not different from the control, suggesting that, in children with TF as in patients with MVD, the decrease of β-AR density is due to a concomitant decrease in β_1- *and* β_2-ARs. However, because of the lack of an appropriate age-matched control group, it cannot be concluded with certainty whether or not β-AR density is decreased in patients with TF.

E. Hypertrophic Obstructive Cardiomyopathy

In membranes from left ventricular septa of 5 patients with HOCM, β-AR density was 28 fmol/mg protein and the β_1-AR to β_2-AR ratio was 80:20% (Golf et al., 1985b), indicating a marked decrease in both β_1- and β_2-ARs. In another study of left ventricular septa, the B_{max} of [^{3}H] DHA or ICYP binding was approximately 72 to 74 fmol/mg protein (with an enormous variability ranging from 20 to 140 fmol/mg protein) in 14 patients with HOCM; the β_1-AR to β_2-AR ratio was not determined (Ferry and Kaumann, 1987). Subsequent studies with [^{3}H]bupranolol as the ligand showed in left ventricular septal tissue of HOCM patients a mean β-AR density between 20 and 60 fmol/mg protein (Kaumann et al., 1989b). No difference was found in right atrial β-AR density between patients with HOCM and patients with other cardiac disorders (coronary artery disease, AVD, MVD, and atrial septal defect) (Wagner et al., 1989). Thus, at present it is uncertain whether and how β-AR density is changed in patients with HOCM, and nearly nothing is known concerning changes in the β_1-AR to β_2-AR ratio.

F. Aortic Valve Disease

In 22 patients with AVD and varying degrees of heart failure (NYHA classes II to IV), right atrial β_1-ARs, but not β_2-ARs, gradually declined with increasing degree of heart failure (Michel et al., 1990). Similar data were reported by Steinfath et al. (1991a,b) for left ventricular papillary muscles of five patients with AVD and advanced heart failure (NYHA classes III to IV): β-AR density was markedly decreased, which was again due to a selective reduction in β_1-ARs (fig. 10). Although it is certainly premature to draw any firm conclusion from only two studies, these data indicate that AVD, like DCM, is a disease associated with a loss of only β_1-ARs.

G. In vivo Alterations of β-Adrenoceptor Function in Chronic Heart Failure

1. Positive inotropic effects. Only a few in vivo studies of alterations of β-AR function have been performed in patients with chronic heart failure. The data reported thus far support the in vitro finding of a reduced β-AR responsiveness.

Comparison of the positive inotropic effect of graded infusions of dobutamine in patients with mild heart failure (left ventricular ejection fraction >0.40) with that in patients with severe heart failure (left ventricular ejection fraction <0.30) mainly due to DCM with biventricular failure showed that the positive inotropic effect of dobutamine was greater in patients with mild heart failure than it was in patients with severe heart failure (fig. 13) (Fowler et al., 1986). On the other hand, the positive inotropic effect of graded infusions of Ca^{2+} (as calcium gluconate) was nearly identical in both patient groups (in good agreement with the in vitro data; see above). Similarly, in studies in which dobutamine was infused into the left main coronary artery in 24 patients with advanced heart failure (NYHA classes III and IV) and in 8 subjects without left ventricular dysfunction, a similar decrease in positive inotropic effect of dobutamine at each dose was found (Colucci et al., 1988). There was an inverse relationship between plasma noradrenaline levels and the magnitude of the positive inotropic effect of dobutamine, indicating that the degree of β-AR desensitization may be linked to the elevation

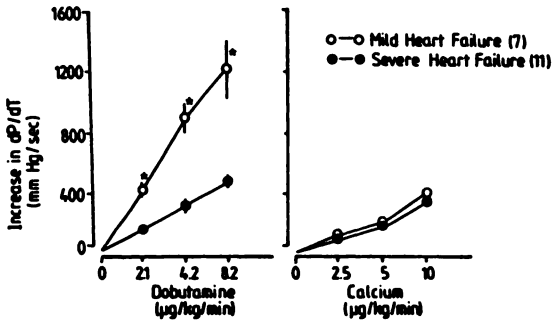

FIG. 13. Net increase in peak positive left ventricular dP/dT in patients with mild (left ventricular ejection fraction > 0.40) and severe (left ventricular ejection fraction < 0.30) heart failure following dobutamine (left) or calcium gluconate (right) infusion. Note that at each dobutamine dose its positive inotropic effect is less in patients with severe than with mild heart failure, whereas no difference in the positive inotropic effect of calcium is observed in the two groups. Bars, ±SEM. *$P < 0.05$ versus the corresponding values in patients with severe heart failure. From Fowler et al. (1986) and used with permission.

of sympathetic activity (if one assumes that plasma noradrenaline levels are an index of overall sympathetic activity).

2. Positive chronotropic effects. As discussed, the two standard tests to study β-AR responsiveness are the heart rate responses to either isoprenaline infusions or dynamic exercise. The chronotropic response to peak exercise is markedly reduced in patients with chronic heart failure (Epstein et al., 1967; Weber et al., 1982; Higginbotham et al., 1983; Francis et al., 1985). This observation can be due to either the impaired baroreceptor function in chronic heart failure (for references, see Hirsch et al., 1987; Rea and Berg, 1990) or the decreased β_1-AR function because exercise-induced tachycardia is, in first approximation, a noradrenaline-induced tachycardia (see section II.C.2.).

Comparison of the plasma noradrenaline increases during exercise in patients with chronic heart failure and in healthy controls showed that the slope in noradrenaline increase with increasing work load was reduced in patients with chronic heart failure (Francis et al., 1982, 1985). The reduced heart rate response to exercise in chronic heart failure may, therefore, result from a reduced sympathetic outflow due to an impairment of baroreceptor function. However, in 37 normal subjects and 51 patients with chronic heart failure (NYHA classes I to IV), peak exercise plasma noradrenaline levels were similar in both groups (Colucci et al., 1989). Nevertheless, during bicycle exercise in patients with chronic heart failure, heart rate at any given plasma noradrenaline level was lower than in normal controls, and, more important, the increase in heart rate was lower for any given increase in plasma noradrenaline. These data indicate that the reduced heart rate response to exercise in chronic heart failure may be due to the impaired cardiac β-AR function in these patients, although a significant contribution of impaired baroreceptor function cannot be excluded.

The heart rate response to isoprenaline infusion also seems to be reduced in patients with chronic heart failure because the increase in heart rate during infusion of isoprenaline was, at any given isoprenaline dose, less than that seen in normal controls (fig. 14; Erne et al., 1988; Colucci et al., 1989). On the other hand, forskolin caused increases in heart rate that were *not* different in patients with chronic heart failure and normal controls (fig. 14) (Erne et al., 1988), which is in good agreement with the in vitro data (see section III.A).

Thus, taken together, the limited data concerning in vivo effects of cardiac β-AR stimulation in chronic heart failure generally agree with the in vitro data that in chronic heart failure β-AR function is reduced. However, the question of whether β_1- or β_2-AR function might be differentially altered in different kinds of heart failure, as has been observed in vitro, is still not answered.

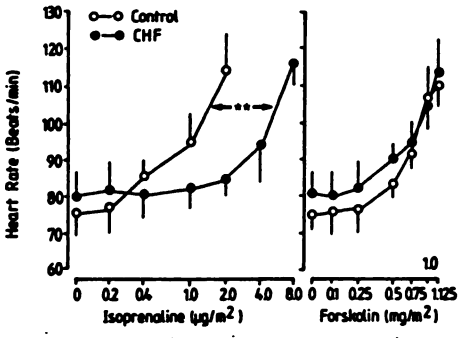

FIG. 14. Heart rate response to increasing doses of bolus injections of isoprenaline (left) and forskolin (right) in six to eight healthy volunteers (control) and six to seven patients with congestive heart failure (CHF; NYHA classes III and IV). $^{**}P < 0.001$ versus control. Bars, SEM. From Erne et al. (1988) and used with permission.

IV. Spare Receptors for β-Adrenoceptor Agonists in the Human Heart?

As discussed, a general feature of cardiac failure in humans is a decrease in the number of β-ARs that is accompanied by a decrease in the functional responsiveness of the heart to β-AR stimulation. Such a concomitant decrease in receptor number *and* in functional responsiveness indicates that in the human heart the maximal positive inotropic effect of catecholamines depends on the number of available β-ARs and that few or no "spare" receptors exist.

The concept of spare receptors (receptor reserve) was introduced into pharmacology by Stephenson (1956) and Nickerson (1956). It described the phenomenon whereby a maximal response to an agonist can be achieved in many tissues with submaximal receptor occupancy. This phenomenon depends on both the tissue and the agonist used. A direct 1:1 relationship between receptor occupancy and response would indicate the absence of spare receptors; when these values differ, e.g., when a 10% receptor occupancy yields a 50% response, the phenomenon is often referred to as receptor reserve. It should be noted, however, that the amount of spare receptors associated with the production of 50% of a response may be quite different from that involved in maximal response. Thus, many examples have been published for which receptor reserve is large for half-maximal responses but smaller or absent for maximal responses (for references, see Furchgott, 1972; Ruffolo, 1982; Kenakin, 1984, 1987). Because of these difficulties, it has been suggested that reference should be made to "linear and nonlinear" stimulus-response relationships rather than to "receptor reserve" (Kenakin, 1987).

The most appropriate method for determination of the receptor reserve for a given drug in a given tissue is to assess the functional response after progressively inactivating the number of functional receptors by an irreversible antagonist. When a receptor reserve is present, the concentration-response curve will initially be shifted to the right without a depression of the maximal response but ultimately will show a depressed maximum at that

point where the minimum number of receptors required to produce a maximal response is reached.

Although phenoxybenzamine has often been used to evaluate the receptor reserve for α-AR agonists (for references, see Furchgott, 1972; Ruffolo, 1982; Kenakin, 1984, 1987), unfortunately, the irreversible β-AR antagonists available at present, bromoacetylalprenololmenthane (BAAM; Pitha et al., 1980) and N^8-(bromoacetyl)-N^1-[3-(4-indolyloxy)-2-hydroxypropyl]-(Z)-1,8-diamino-*p*-menthane (BIM; Pitha et al., 1987), have not been used for studies of a receptor reserve for β-AR agonist-induced positive inotropic effects in the human heart.

BAAM has been used to block AC activation in left ventricular membranes from nonfailing and failing hearts (Port and Bristow, 1988). A direct relationship was found between removal of β-ARs and decrease in AC activation by isoprenaline, indicating that no receptor reserve for β-AR agonists exists in the human left ventricle. However, experiments with isoprenaline and adrenaline in human right atrial membranes show that a 1:1 relationship exists between fractional receptor occupancy and percentage of AC activation at each agonist concentration (Brown et al., 1991); the same holds true even in a tissue with a large receptor reserve such as the cat heart (Kaumann, 1978; Kaumann et al., 1989b). This approach, therefore, cannot give any information concerning receptor reserve for β-AR agonist-induced responses.

Another approach involved simultaneous determination of β-AR B_{max} by [^{3}H]CGP 12177 binding and maximal inotropic responses to isoprenaline stimulation in left papillary muscles from three groups of patients with different degrees of heart failure (Schwinger et al., 1990): nonfailing hearts, patients with MVD and mild to moderate heart failure (NYHA classes II to III), and patients undergoing heart transplantation due to end-stage DCM (NYHA class IV). A linear relationship was found between B_{max} and the maximal inotropic response to isoprenaline (fig. 15), leading to the conclusion that there are no spare receptors for isoprenaline in the human ventricular myocardium. However, no attempt was made to calculate fractional receptor occupancy and response; hence, the question of whether under these conditions the stimulus-response relationship is linear (as to be expected for "virtually no receptor reserve") or nonlinear has not been addressed. Moreover, there are many cases showing a poor correlation between receptor density (as determined by radioligand-binding studies, which determine all β-ARs in a crude membrane fraction) and response. Receptor density, as assessed by radioligand-binding techniques, therefore, does not necessarily relate to receptor responsiveness (for references, see Kenakin, 1984, 1987).

The above data, therefore, suggest that the human ventricular myocardium appears to contain only a small

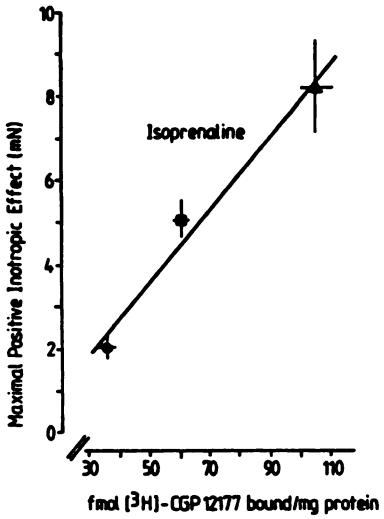

FIG. 15. Correlation between left ventricular β-AR density (assessed by Scatchard (1949) analysis of [^{3}H]CGP 12177 binding) and maximal positive inotropic effects induced by isoprenaline on isolated electrically driven left papillary muscles from nonfailing hearts (▲; n = 5), patients with mild to moderate heart failure (NYHA classes II to III; ■; n = 10), and patients with end-stage DCM (NYHA class IV; ●; n = 16). Bars, ±SEM. From Schwinger et al. (1990) and used with permission.

receptor reserve for isoprenaline but certainly cannot finally answer this question.

A more appropriate approach to study the possible existence of spare receptors for β-AR-mediated inotropic effects in the human heart is to determine on isolated electrically driven human cardiac preparations the positive inotropic effects of catecholamines and, simultaneously, their affinities for β_1- and β_2-ARs by radioligand-binding studies. From these data plots can be constructed of fractional receptor occupancy versus response.

It should be kept in mind, however, that the critical point of this approach is the determination of the affinities of the catecholamines at the β-ARs, especially because it is still an open question which affinity state of the β-ARs (the high, GTP-sensitive or the low, GTP-insensitive state) would be relevant to the functional response.

It can be shown with this approach that in the human right atrium and left papillary muscles spare receptors exist for positive inotropic responses to the nonselective β-AR agonist adrenaline (Kaumann et al., 1989b; Hall et al., 1990). A plot of fractional receptor occupancy versus response in right atria from patients undergoing elective coronary artery bypass grafting ("nearly normal hearts") using the value for affinity of 2 μM and an EC_{50} value of 0.17 μM (Hall et al., 1990) revealed that adrenaline has to occupy 8% of β-ARs to cause 50% of the maximal inotropic response and 45% of β-ARs to cause 90% of the maximal response. In left papillary muscles from patients with MVD, 50% of the maximal inotropic effects of adrenaline can be obtained at approximately 14% of β-AR occupancy (Kaumann et al., 1989b). In contrast,

in the rat left atrium (cf. fig. 16), right and left ventricular papillary muscles (Brodde et al., 1990a; Brown et al., 1991) and in cat left ventricular papillary muscles (Kaumann et al., 1989b), isoprenaline and adrenaline have to occupy only 1.5 to 3% of the receptors to cause 50%, and approximately 20% to cause 90%, of maximal response.

These data seem to indicate that (*a*) there are spare receptors for the positive inotropic responses to adrenaline in the human heart, (*b*) they are present in much smaller amounts than those found in the rat or cat heart, and (*c*) there are fewer spare receptors in left ventricles than in right atria. However, patients with MVD usually have a moderate degree of heart failure and a decreased number of β-ARs (see section III.C) which could explain the fewer spare receptors in left ventricles compared to right atria. In fact, a plot of fractional receptor occupancy versus response in left papillary muscles from nonfailing hearts using published data for isoprenaline [EC_{50} 16 nM (Schwinger et al., 1990)] and an affinity for human cardiac β-ARs of 178 nM (cf. fig. 16) reveals that in the nonfailing human left papillary muscle isoprenaline has to occupy 8 to 10% of β-ARs to cause 50% of the maximal inotropic response, which is comparable with values obtained for adrenaline in the right atrium. These data also indicate that with increasing degree of heart failure not only the number of β-ARs decreases but also the amount of spare receptors.

In a recent study this hypothesis was investigated directly (Brodde et al., 1990a; Brown et al., 1991). In right atria from patients with AVD and different degrees of heart failure (NYHA classes II to IV), the positive inotropic effects of isoprenaline were determined. Simultaneously, the affinity of isoprenaline for right atrial β-ARs was assessed by ICYP binding. To avoid the problem of binding of the agonist to two affinity states of the β-ARs, and to mimic the organ bath conditions as closely as possible, ICYP binding was performed in a Na^+-rich phosphate-buffered Krebs-Henseleit solution as incubation medium (as originally suggested by McPherson et al., 1984, 1985). Under these conditions isoprenaline binds to a homogeneous population of β-ARs, because competition curves with pseudo-Hill coefficients not significantly different from unity were obtained, and GTP had no significant influence on the slope of the curves (Brodde et al., 1990a; Brown et al., 1991). In right atria from patients with NYHA class II, isoprenaline had to occupy approximately 8% of the β-ARs to produce 50% and approximately 44% to produce 90% of the maximal response (fig. 16). However, with increasing degree of heart failure (from NYHA class II to class IV), the proportion of receptors to be occupied to induce 50% of the maximal response gradually increased and in patients with NYHA class IV an almost 1:1 relation between receptor occupancy and response was obtained (fig. 16).

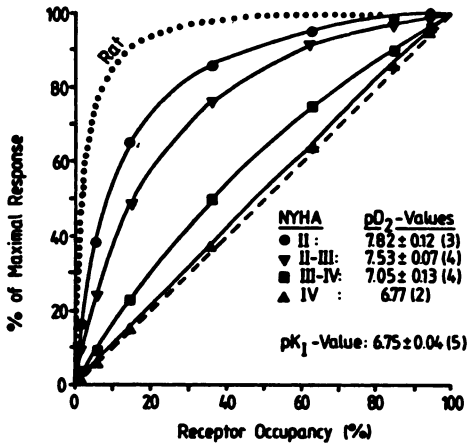

FIG. 16. Plots of percentage of receptor occupancy versus positive inotropic effect of isoprenaline on isolated electrically driven right atria from patients with AVD and different degrees of heart failure (judged clinically by NYHA functional class). Ordinate, Positive inotropic effect of isoprenaline in percentage of maximal response. For each group of patients the maximal response was set to 100%; abscissa, receptor occupancy in percentage, calculated from the pK_I value of isoprenaline (assessed by ICYP binding). For details see text. -----, line of identity. For comparison, data for rat left atria (· · · · ·) [pD_2 = 8.49 ± 0.07 (n = 4); pK_I = 6.74 ± 0.06 (n = 6)] are included. From Brodde et al. (1990a).

Similar results were obtained in left ventricular papillary muscles obtained from patients with MVD and patients with DCM and different degrees of heart failure (NYHA classes II to III to class IV). The β_1-AR-mediated positive inotropic effect of isoprenaline (30 nM ICI 118,551 used to block β_2-ARs) was determined together with the affinity of isoprenaline for left ventricular β_1-ARs (ICYP binding in the presence of 50 nM ICI 118,551) (Brodde et al., 1990a; Brown et al., 1991). In patients with NYHA classes II to III approximately 20 to 25% of β_1-ARs had to be occupied to reach 50% of the maximal inotropic response, a figure that is comparable to that obtained with adrenaline by Kaumann et al. (1989b). However, with an increasing degree of heart failure the proportion of receptors needed to be occupied increased and in patients with DCM and NYHA class IV there was again a 1:1 ratio between receptor occupancy and response.

Thus, taken together, in the human heart there is a small receptor reserve for the inotropic responses to the full agonists isoprenaline and adrenaline (at least to reach 50% of maximal response); however, nearly the total receptor fraction has to be occupied to achieve a maximal response. In heart failure, β-AR density declines together with the spare receptors so that in end-stage heart failure (NYHA class IV) virtually each receptor is needed to bring about a response.

Nothing is known at present about spare receptors for positive chronotropic effects of β-AR agonists in the human heart.

V. β-Adrenoceptors in the Transplanted Human Heart

It is a general phenomenon that after long-term withdrawal of endogenous catecholamines from the β-ARs

(either by denervation or by long-term receptor blockade) the subsequent response to agonists is increased, i.e., a reduced amount of β-AR agonist is required to produce the same response as in a normal state (for references, see Trendelenburg 1963, 1966; Broadley et al., 1984). Such a supersensitivity is often accompanied by an increased number of functional β-ARs (for references, see Stiles et al., 1984; Lefkowitz and Caron, 1985; Brodde, 1989). The transplanted human heart is a denervated organ (Beck et al., 1969; Cannom et al., 1973; Mason et al., 1976, Bristow, 1990) with drastically reduced tissue noradrenaline (Port et al., 1990a; Regitz et al., 1990; Bristow, 1990). Thus, it might develop an up-regulation of β-ARs and/or a supersensitivity of the β-ARs to β-AR stimulation. Studies in animal models of cardiac transplantation or experimentally induced denervation show an increase in myocardial β-ARs (Lurie et al., 1983; Vatner et al., 1985). In two studies of patients after transplantation an increased chronotropic response to isoprenaline infusion was observed (Borow et al., 1985; Yusuf et al., 1987). However, these studies were performed without prior atropine treatment. Because isoprenaline infusion actually increases vagal tone (see section II.C.2), thus blunting its effect, the difference between cardiac transplant recipients and healthy controls in the response to isoprenaline could be due to attenuation of the response in healthy volunteers but *not* in the patients. In fact, when heart transplant recipients are pretreated with atropine, the chronotropic effect of isoprenaline was not different between the native (innervated) and transplanted (denervated) atrium (Gilbert et al., 1989). However, even after atropine treatment the chronotropic response to adrenaline was greater in the native (innervated) than in transplanted (denervated) atrium (Gilbert et al., 1989). The reason for this difference is not completely understood but may be due to the fact that adrenaline is taken up into sympathetic nerve terminals in the normal, but not in the transplanted, heart. Hence, at each dose the concentration of adrenaline in the synaptic cleft and at the receptor is higher in transplanted than in normal hearts, leading to an enhanced response.

Some data concerning numbers of β-ARs in the transplanted human heart are available. In their first study Bristow and associates (Gilbert et al., 1989) observed that with increasing posttransplantation time (up to 100 days) numbers of β-ARs decreased. Subsequently, this group, however, showed that in previously transplanted hearts from patients with normal cardiac function undergoing retransplantation because of graft atherosclerosis (average posttransplantation time 23 months) numbers of left ventricular β-ARs were not different from those in nonfailing hearts (Port et al., 1990a). This has been confirmed in right ventricular endomyocardial biopsies from cardiac transplant recipients (average posttransplantation time 9 months) which had β-AR levels not different from those in normal hearts (Denniss et al., 1989b). Serial measurements of the development of β-ARs in right ventricular endomyocardial biopsies of heart transplant recipients in weekly and, later, monthly intervals for 6 to 18 posttransplantation months showed that numbers of β-ARs in the biopsies were significantly higher than in ventricular membranes of the explanted native hearts; during this whole period numbers of β-ARs in the biopsies were quite stable and did not significantly change (Brodde et al., 1991b). On the other hand, with increasing posttransplantation time the right ventricular endomyocardial β_1-AR to β_2-AR ratio appears to decline from 80:20% initially to 66:33% after 18 to 23 months (Port et al., 1990b; Brodde et al., 1991b).

Very few in vitro functional studies of β-ARs in the transplanted heart have been published. They show a reduced responsiveness of right ventricular endomyocardial AC to stimulation with Gpp(NH)p and isoprenaline (Denniss et al., 1989b) or, in contrast, an enhanced response of right ventricular endomyocardial AC to isoprenaline and the β_2-AR agonist terbutaline, but no changes in NaF-evoked (i.e., G_s-mediated) AC activation (Bjornerheim et al., 1987; Port et al., 1990b).

In isolated right ventricular trabeculae from previously transplanted hearts (see above), the maximal positive inotropic effects to isoprenaline (β_1- and β_2-ARs) and zinterol (β_2-AR) were not different from those obtained in nonfailing hearts (Port et al., 1990a). On the other hand, because tissue noradrenaline is low in the transplanted heart, it is not surprising that the effects of the directly and indirectly acting β-AR agonists, dopamine (via release of noradrenaline) and dopexamine (via inhibition of uptake), were markedly attenuated (fig. 17) (Port et al., 1990a).

In summary, the limited data available indicate that the transplanted human heart shows few (if any) signs of postsynaptic β-AR supersensitivity or increased numbers of β-ARs.

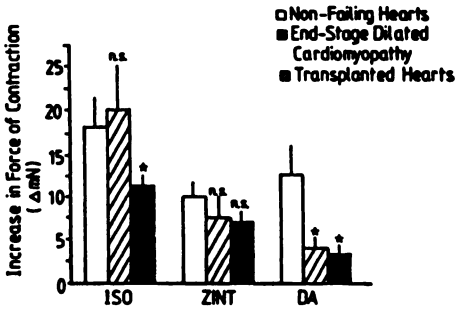

FIG. 17. Maximal positive inotropic effect of β-AR agonists on isolated electrically driven right ventricular trabeculae from nonfailing hearts, previously transplanted (denervated) hearts, and hearts derived from patients with end-stage DCM. Bars, SEM. *$P < 0.05$ versus the corresponding values in nonfailing hearts; n.s., not significantly different from the corresponding values in nonfailing hearts. ISO, isoprenaline; ZINT, zinterol; DA, dopamine (in the absence of neuronal uptake blockade). From Port et al. (1990a) and used with permission.

VI. Effects of Drugs on β_1- and β_2-Adrenoceptors in the Human Heart

A. β-Adrenoceptor Agonists

As described, a common feature of heart failure in humans is a decrease in numbers of β-ARs and, because there are few spare receptors, a concomitant decrease in β-AR responsiveness. Thus, one possible approach to give the failing human heart inotropic support could be through the use of β-AR agonists that possess positive inotropic effects. However, because it is a general phenomenon that long-term application of agonists leads to a desensitization and finally to a down-regulation of β-ARs (Harden, 1983; Stiles et al., 1984; Hertel and Perkins, 1984; Lefkowitz and Caron, 1985; Hausdorff et al., 1990), the use of β-AR agonists in patients with severe heart failure should be of limited value because tolerance to their action will develop (Colucci et al., 1981, 1986a; Leier et al., 1978; Unverferth et al., 1980; Lambertz et al., 1984; Erlemeier et al., 1986).

A down-regulation of β-ARs in humans following continuous β-adrenergic activation has been directly shown in women undergoing β_2-adrenergic tocolytic therapy; numbers of myometrial β-ARs were markedly depressed when compared with nontreated women (Berg et al., 1985; Michel et al., 1989). In healthy volunteers treated for 9 days with the β_2-AR agonist procaterol, *all* β_2-AR-mediated physiological effects (i.e., isoprenaline infusion-induced decrease in diastolic blood pressure and increase in plasma noradrenaline levels) were markedly attenuated, whereas β_1-AR-mediated effects (isoprenaline-induced increase in systolic blood pressure and exercise-induced tachycardia) were unchanged (Brodde et al., 1990b). Isoprenaline-induced tachycardia involving both β_1- and β_2-ARs also was attenuated but to a lesser extent than were the pure β_2-AR-mediated effects. Similarly, after 14 days of treatment with xamoterol (selective β_1-AR partial agonist) all β_1-AR-mediated, but *not* β_2-AR-mediated, effects were attenuated. Again, isoprenaline-induced tachycardia was attenuated but less than the pure β_1-AR-mediated effects (Brodde et al., 1990b).

Thus, long-term treatment with a subtype-selective β-AR agonist causes subtype-selective desensitization. As discussed, the human heart has only a small receptor reserve for β-AR agonists which is further reduced in heart failure. Furthermore, in all kinds of heart failure β_1-AR function is always depressed, whereas β_2-AR function varies. It can be predicted, therefore, that, if β-AR-mediated positive inotropic support is needed, nonselective full agonists should be most effective. Partial agonists should be less effective because their effects strongly depend on the numbers of β-ARs available. Indirect sympathomimetics will not be very effective, because noradrenaline stores are depleted. Thus, isoprenaline, adrenaline, and dobutamine (and perhaps epinine) could be the drugs of choice, whereas dopamine, dopexamine, denopamine, or xamoterol should be not very effective. On the other hand, full agonists cause a more rapid desensitization than do partial agonists (the latter compounds, in chronic heart failure with high sympathetic drive, may actually act as antagonists). Thus, in general, the use of β-AR agonists in long-term treatment of chronic heart failure may be of limited value.

B. β-Adrenoceptor Antagonists

Decreased β-AR function in chronic heart failure might be caused by "endogenous down-regulation" through enhanced (cardiac derived) catecholamines (Bristow et al., 1988). To ameliorate these effects and to improve ventricular performance, attempts have been made to treat patients suffering from DCM with β-AR antagonists (Waagstein et al., 1975, 1983, 1989; Swedberg et al., 1979, 1980; Ikram and Fitzpatrick, 1981; Currie et al., 1984; Anderson et al., 1985; Engelmeier et al., 1985; Heilbrunn et al., 1989; Gilbert et al., 1990; Eichhorn et al., 1990), because, in the rat heart (Glaubiger and Lefkowitz, 1977; Aarons and Molinoff, 1982), lung, and lymphocytes (Aarons and Molinoff, 1982) as well as in human lymphocytes (Aarons et al., 1980; Fraser et al., 1981; Brodde et al., 1985; Whyte et al., 1987; van den Meiracker et al., 1987, 1989), chronic administration of propranolol (a nonselective β-AR antagonist without PAA) produces a substantial increase in β-AR density.

A few studies have been conducted in which the effects of chronic β-AR antagonist treatment on β-ARs were examined in the human heart. Baumann et al. (1983) observed in two patients with MVD who had been treated for 10 months with metoprolol that the numbers of left ventricular β-ARs were markedly higher than in nontreated patients with MVD. In seven children with TF the effects of chronic propranolol treatment on right atrial and right ventricular β-AR density were studied; in both tissues β_1-AR and β_2-AR density tended to be higher than in nontreated patients (Brodde et al., 1989b). In four patients undergoing elective coronary artery bypass grafting, chronic treatment with the nonselective β-AR antagonists propranolol or sotalol significantly increased right atrial β_1- and β_2-AR densities (Jones et al., 1990). Similarly, chronic treatment with propranolol, atenolol, metoprolol, or timolol increased the numbers of β-ARs in right atria from patients undergoing elective coronary artery bypass grafting (Hedberg et al., 1985; Wagner et al., 1989). Unfortunately, in these latter studies no attempt was made to analyze whether the increases in numbers of β_1- or β_2-ARs were different in patients treated with the selective β_1-AR antagonists atenolol or metoprolol compared with those induced by the nonselective β-AR antagonists propranolol or timolol. Such an analysis has been performed in the study of Michel et al. (1988) in 44 patients undergoing elective coronary artery bypass grafting; 34 of these patients were treated chronically with the β_1-AR-selective antagonists atenolol or metoprolol, the nonselective β-AR antagonists without PAA propranolol or sotalol, or the nonselective β-AR

antagonist pindolol with PAA. In the patients treated with the β_1-AR-selective antagonists, numbers of right atrial β_1-ARs were significantly increased (fig. 18), whereas β_2-ARs were only increased in the patients treated with the nonselective β-AR antagonists propranolol or sotalol. The number of β_2-ARs was unchanged in the patients treated with atenolol or metoprolol and even decreased in the patients receiving pindolol [which possesses in many tissues a "β_2-AR-selective PAA" that causes a selective down-regulation of β_2-ARs (Giudicelli et al., 1984; Neve et al., 1985; Hedberg et al., 1986; Brodde et al., 1986b; van den Meiracker et al., 1987, 1989)]. This effect of chronic administration of pindolol on human right atrial β_1- and β_2-ARs has been subsequently confirmed (Bjornerheim et al., 1990).

Surprisingly, the increase in numbers of right atrial β_1-ARs following chronic treatment with β_1-AR-selective antagonists is not accompanied by an increased β_1-AR-mediated positive inotropic effect but is associated with a markedly enhanced β_2-AR-mediated effect. This was first shown by Hall et al. (1988) who found that, in right atria from patients chronically treated with atenolol, the positive inotropic effect of noradrenaline via β_1-AR activation, despite an increased number of β_1-ARs, was not changed, whereas the inotropic action of adrenaline mediated through activation of both β_1- *and* β_2-ARs was markedly enhanced. Moreover, the β_2-AR-mediated positive inotropic effect of salbutamol, that amounted in non-β-AR antagonist-treated patients to approximately 39% of that of isoprenaline, was markedly enhanced in atenolol-treated patients; the maximal inotropic effect increased to 80% of that of the response to isoprenaline (Hall et al., 1990). Nearly identical results were obtained with the selective β_2-AR agonist procaterol (fig. 19): in non-β-AR antagonist-treated patients it usually produced 75 to 80% of the response of isoprenaline, whereas in patients chronically treated with the selective β_1-AR antagonists metoprolol, atenolol, or bisoprolol it produced identical maximal responses to isoprenaline (Motomura et al., 1990a). Thus, it seems that in human right atria chronic β_1-AR antagonist treatment causes a selective up-regulation of β_1-ARs but a sensitization of β_2-ARs. There is some preliminary indication that a similar β_2-AR-sensitizing effect of chronic β_1-AR antagonist treatment also can be demonstrated in vivo. Analysis of the effects of acute and chronic (14 days) treatment of healthy volunteers with the β_1-AR antagonist bisoprolol and the β_2-AR antagonist ICI 118,551 (both given in doses that produced comparable β_1- and β_2-AR occupancies, respectively) on isoprenaline infusion-induced tachycardia (which in humans is mediated equally by β_1- and β_2-ARs; see section II.C.2) reveals that acute β_1-AR blockade caused an approximately threefold larger rightward shift of the isoprenaline dose-response curve than did chronic β_1-AR blockade, whereas the effects of acute and chronic β_2-AR blockade were similar (Brodde et al., 1988; Motomura et al., 1990b). Furthermore, in patients with chronic stable angina who have been chronically treated with atenolol, the positive chronotropic effect induced by intracoronary injection of increasing doses of salbutamol appears to be enhanced, when compared with that in non-β_1-AR antagonist-treated patients (J. A. Hall, M. C. Petch, and M. J. Brown, personal communication).

The mechanism underlying this phenomenon is not clear at present. It is not due to enhanced activation of protein kinase A, because dibutyryl cAMP caused the

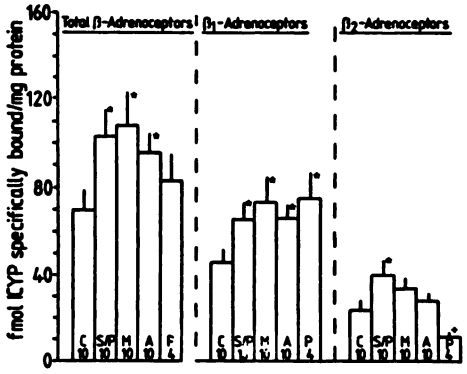

FIG. 18. Effects of chronic treatment with different β-AR antagonists on total β-, β_1-, and β_2-AR density in right atria derived from patients without apparent heart failure undergoing elective coronary artery bypass grafting. Ordinate, Total β-, β_1-, and β_2-AR density in right atrial membranes in fmol ICYP specifically bound/mg protein. Columns, means; bars, SEM; numbers in columns, numbers of experiments. C, Control (i.e., patients *not* treated with β-AR antagonists); S/P, sotalol/propranolol; M, metoprolol; A, atenolol; P, pindolol. $^*P < 0.05$, +, $0.1 > P > 0.05$ versus the corresponding values in control. From Michel et al. (1988) and used with permission.

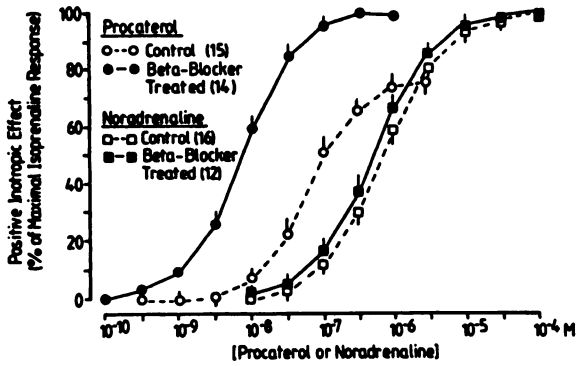

FIG. 19. Positive inotropic effects of procaterol and noradrenaline in isolated electrically driven right atria from patients without apparent heart failure undergoing elective coronary artery bypass grafting; these patients had been chronically treated with β_1-AR-selective antagonists (metoprolol, atenolol, bisoprolol) in comparison to nontreated patients ("control"). The positive inotropic effect is given as percentage of maximal positive inotropic effect induced by isoprenaline. Note that in nontreated patients procaterol caused only 75% of maximal positive inotropic effect of isoprenaline; in β_1-AR antagonist-treated patients the concentration-response curve of procaterol is shifted to the left and the maximal positive inotropic effect is not different from that of isoprenaline. On the other hand, the positive inotropic effect of noradrenaline is nearly identical in both patient groups. Bars, SEM. Modified from Motomura et al. (1990a) and used with permission.

same maximal positive inotropic effect in nontreated and atenolol-treated atria (Hall et al., 1990). Similarly, the positive inotropic effects evoked by Ca^{2+} were not different in treated and nontreated atria (Hall et al., 1990).

Another possible explanation could involve changes in the amount and/or activity of G_s and G_i proteins in the human right atria following chronic β_1-AR antagonist treatment. β_1-AR antagonist treatment might cause prevention of a tonic down-regulation of G_s protein, maintained by endogenous catecholamines, thereby resulting in enhanced G_s function. However, in propranolol-treated patients right atrial messenger RNA levels for G_s protein are unaltered (Jones et al., 1990). Determination of G_i levels in right atria from nontreated and β_1-AR antagonist-treated patients through the use of pertussis toxin-catalyzed ADP ribosylation shows no differences in the levels of G_i in either treated or nontreated patients (Brodde et al., 1991c). Thus, the mechanism underlying this β_2-AR-sensitizing process by β_1-AR antagonists remains to be elucidated.

Recently, two studies have shown that, in patients with DCM, long-term treatment with β-AR antagonists caused a significant increase in β-AR density (fig. 20) as assessed in right ventricular endomyocardial biopsy samples (Heilbrunn et al., 1989; Waagstein et al, 1989). In both studies β-AR density increased by approximately 50 to 100% after 6 to 12 months of treatment with increasing doses of metoprolol; this was accompanied by a marked improvement of cardiac hemodynamics. In addition, the increase in the number of β-ARs was accompanied by a significant increase in the positive inotropic response to dobutamine infusion (Heilbrunn et al., 1989) (fig. 21). Unfortunately, in both studies the β_1-AR to β_2-AR ratio was not determined; thus, it remains an open question whether long-term treatment with β_1-AR antagonists in the human ventricular myocardium

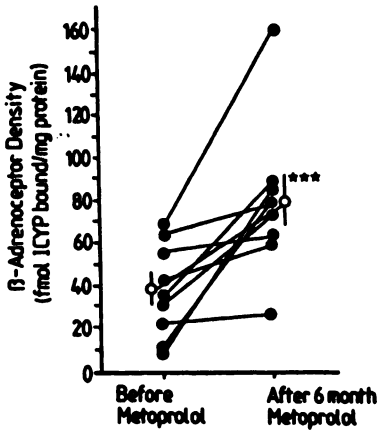

FIG. 20. Effect of 6 months of metoprolol treatment on β-AR density in right ventricular endomyocardial biopsy specimens derived from 9 patients with DCM (NYHA classes I to III). Bars, ±SEM. ***The mean β-AR density in right ventricular endomyocardial biopsies is after 6 months of metoprolol treatment significantly higher ($P < 0.005$) than it was before metoprolol treatment. From Heilbrunn et al. (1989) and used with permission.

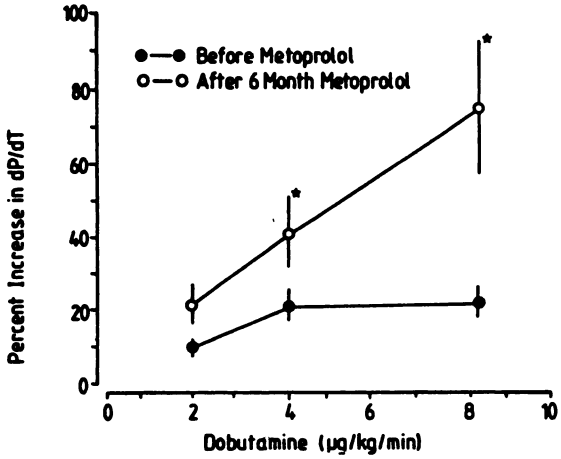

FIG. 21. Percentage of increment in peak left ventricular dP/dT induced by dobutamine infusion in eight patients with DCM (NYHA classes I to III) before and after 6 months of treatment with metoprolol. *The increase in peak left ventricular dP/dT is after 6 months of metoprolol treatment significantly higher ($P < 0.05$) than it was before metoprolol treatment. Bars, ±SEM. From Heilbrunn et al. (1989) and used with permission.

also might increase the number of β_1-ARs, while simultaneously sensitizing β_2-AR function. If such a change occurred, it would suggest the intriguing possibility that an additional intermittent pulsatile treatment with a β_2-AR agonist might further improve cardiac performance. Such an improvement in cardiac function following infusions of β_2-AR selective agonists such as fenoterol, salbutamol, or pirbuterol has been demonstrated in patients with advanced heart failure (Sharma and Goodwin, 1978; Bourdillon et al., 1980; Colucci et al., 1981; Irmer et al., 1981; editorial in the Lancet, 1983; Vik-Mo et al., 1987).

C. Calcium Antagonists

In recent years evidence has accumulated that in the heart the number of β-ARs and the number of L-type calcium channels may be coregulated. In patients with HOCM, ventricular β-AR density and the density of calcium channels (determined by [^{3}H]nimodipine binding) was significantly correlated (Ferry and Kaumann, 1987). In cultured chick embryo ventricular cells, a 4-h exposure of the cells to 1 μM isoprenaline caused a decrease in both the number of β-ARs and calcium channels (assessed by [^{3}H](+)-PN 200-110 binding; Marsh, 1989). Moreover, the numbers of both β-ARs and calcium channels in the rat heart were found to be increased following 6-hydroxy-dopamine treatment (Skattebol and Triggle, 1986). In addition, CEBs may modulate the number of β-ARs. Incubation of cultured cardiac myocytes isolated from the neonatal rat ventricle with different CEBs (verapamil, diltiazem, and nicardipine) produced a time- and concentration-dependent increase in the number of β-ARs (Yonemochi et al., 1990). In vitro experiments have shown that CEBs (nifedipine, diltiazem, and verapamil) might prevent isoprenaline-

induced desensitization of β_2-ARs in circulating human lymphocytes (Hui and Yu, 1988, 1989).

Few studies have been performed on the effects of chronic CEB treatment on the number of β-ARs and subtype distribution in the human heart. In right atria derived from patients undergoing elective coronary artery bypass grafting, chronic treatment with nifedipine produced a significant increase in β-ARs (Hedberg et al., 1985. Conversely, other reports showed that in the same tissue (Jones et al., 1990; Brodde et al., 1991a) and in right atria from patients with HOCM (Wagner et al., 1989) chronic treatment with different CEBs (nifedipine, diltiazem, and verapamil) had no influence on right atrial β-AR density and the increase in β-AR density caused by chronic β-AR antagonist therapy was not affected by simultaneous application of the CEB (Jones et al., 1990; Brodde et al., 1991a). Moreover, chronic CEB treatment also did not prevent the decrease in right atrial β-AR density seen in patients with MVD and moderate heart failure (NYHA class III to classes III to IV; Brodde et al., 1991a).

D. Angiotensin-converting Enzyme Inhibitors

Angiotensin II facilitates noradrenaline release (for references, see Starke, 1977; Westfall, 1977; Langer, 1980), and circulating levels are increased in chronic heart failure, leading to the speculation that systemic or local ACE inhibition might have an antiadrenergic effect by withdrawal of facilitation of noradrenaline release. In chronic heart failure ACE inhibitors tend to decrease plasma noradrenaline levels (for references, see Kubo, 1990). Such an effect might also occur locally in the heart, because the existence of a cardiac renin-angiotensin system has been convincingly demonstrated (Becker et al., 1989; Mebazaa et al., 1989; Urata et al., 1990).

Because β-AR down-regulation in heart failure might be caused by an increase in (locally in the heart) released endogenous catecholamines (Bristow et al., 1988), chronic ACE inhibition may help to restore, at least partly, receptor number and function. Results of few studies have been published in which this hypothesis was tested. In guinea pigs, chronically treated with isoprenaline to mimic chronic β-AR stimulation seen in heart failure, cardiac β-AR density is markedly decreased (Maisel et al., 1989b). The decrease in cardiac β-ARs, but *not* in β_2-ARs in lymphocytes, could be prevented by the simultaneous application of captopril in these animals (Maisel et al., 1989b). However, in lymphocytes from patients with congestive heart failure who had decreased β-AR density and G_s protein levels (as assessed by cholera toxin-catalyzed ADP ribosylation), captopril, or lisinopril caused an up-regulation of both parameters (Horn et al., 1988). In one study of patients with heart failure, β-AR in right ventricular endomyocardial biopsies were increased by lisinopril treatment which also improved hemodynamics of the patients (Gilbert et al., 1988).

E. Phosphodiesterase Inhibitors

PDE inhibitors are another class of positive inotropic agents for the treatment of chronic heart failure (Colucci et al., 1986b). They probably exert their effects by inhibiting the breakdown of cAMP, thereby enhancing the intracellular level of cardiac cAMP (Schmitz et al., 1989b). In nonfailing human ventricular trabeculae or papillary muscles, PDE inhibitors such as isobutylmethylxanthine (Feldman et al., 1987; Schmitz et al., 1987; Näbauer et al., 1988; Böhm et al., 1988a), milrinone (Feldman et al., 1987; Böhm et al., 1988c), pimobendan, adibendan, and saterinone (Von der Leyen et al., 1991) effectively increase contractile force; in failing hearts, however, the effects are blunted. If cAMP levels are elevated by a minimally effective concentration of forskolin the positive inotropic effects of milrinone and isobutylmethylxanthine were partially restored (Feldman et al., 1987). Similarly, isoprenaline (0.1 or 0.2 μM to increase cAMP levels via β-AR stimulation) enhances the effectiveness of several PDE inhibitors on isolated electrically driven ventricular trabeculae or papillary muscles from patients with severe heart failure (Böhm et al., 1988c; Von der Leyen, 1991). Because the positive inotropic effect of PDE inhibitors strongly depends on the increase in intracellular cAMP (Gristwood et al., 1987; Schmitz et al., 1989b; Von der Leyen et al., 1991), this indicates that an abnormality in cAMP production exists in the failing human heart.

The effect of long-term treatment with PDE inhibitors on cardiac β-ARs is not completely understood. In patients with DCM, enoximone only marginally affected total β-AR density but did shift the β_1-AR to β_2-AR ratio toward β_2-ARs (Feldman and Bristow, 1990). Thus, the number of β_1-ARs was decreased, whereas the number of β_2-ARs was increased. The mechanism underlying these differential changes in β_1- and β_2-ARs in the human ventricular myocardium is presently not known. In contrast, animal studies have shown that PDE inhibitor administration can decrease total β-AR density possibly by heterologous desensitization caused by the increased cAMP levels (Bobik and Little, 1984). Also, in patients with decompensated class II or IV heart failure, a continuous infusion of the PDE inhibitor amrinone led to a marked decrease in lymphocyte β_2-AR density (Maisel et al., 1989c). This effect was accompanied by a waning of the hemodynamic improvement, initially observed, after 36 to 72 h of continuous treatment.

VII. Conclusion

There can be no doubt that functional β_1- and β_2-ARs exist in the human heart and that both couple to AC with the β_2-AR subtype being more efficiently coupled. Both β_1- and β_2-ARs mediate the positive inotropic and chronotropic effects to β-AR agonists. β_1-AR stimulation causes maximal positive inotropic effects both in atria and ventricles, whereas β_2-AR stimulation induces max-

imal positive inotropic effects only in atria but submaximal responses in ventricles. Among the endogenous catecholamines, noradrenaline acts nearly exclusively at cardiac β_1-ARs, whereas adrenaline stimulates both β_1- and β_2-AR equally. Because noradrenaline is the major peripheral sympathetic neurotransmitter in humans, it is likely that, under normal physiological conditions, only β_1-ARs regulate heart rate and contractility. In situations of stress, however, when large amounts of adrenaline are released from the adrenal medulla, additional cardiac β_2-AR stimulation can contribute to increases in heart rate or contractility or both.

Whether β_3-ARs, which have been recently cloned (Emorine et al., 1989) and may or may not be identical with the atypical β-ARs found in adipocytes (Zaagsma and Nahorski, 1990), exist in the human heart is not known at present. The existence of a third β-AR in the heart has been suggested recently based on the findings that, in guinea pig and cat hearts, "nonconventional" β-AR antagonists with PAA (e.g., pindolol and chemically derived analogues of pindolol) exhibit, unlike classical β-AR antagonists with PAA, stimulant activities only at concentrations greatly exceeding those required for β-AR blockade (Kaumann, 1989). These stimulant effects are quite resistant to β_1- or β_2-AR-saturating concentrations of β-AR antagonists. Although in vitro (−)-pindolol does not evoke positive inotropic effects in isolated human right atria or left ventricles (Kaumann and Lobnig, 1986), in vivo racemic pindolol can cause sinoatrial tachycardia in humans (van den Meiracker et al., 1987). Thus, the possibility cannot be ruled out that a third sinoatrial β-AR mediating chronotropic effects may exist in the human heart.

In chronic heart failure cardiac β-AR density and responsiveness is reduced, and this loss in cardiac β-AR function is strongly related to the degree of heart failure (as evaluated by NYHA functional class). However, β_1- and β_2-ARs are differentially changed in different forms of heart failure. In DCM and possibly in AVD, the number of cardiac β_1-ARs is selectively reduced without alteration in the number of β_2-ARs (although β_2-ARs become somewhat uncoupled). Under these conditions cardiac β_2-ARs may, at least partially, compensate for the loss of β_1-ARs, maintaining contractility.

In ICM, MVD, and TF both β_1- *and* β_2-AR density and responsiveness are concomitantly decreased. The pathophysiological mechanisms underlying these differential changes are not presently understood. They may reflect different changes in endogenous catecholamines; therefore, in DCM there may be a selective increase in noradrenaline (Francis, 1985) which may selectively down-regulate cardiac β_1-ARs. Such a selective β_1-AR down-regulation has been observed in rats in which a noradrenaline-secreting pheochromocytoma was implanted, and only β_1-ARs, but *not* β_2-ARs, were reduced in several tissues (Snavely et al., 1982; Tsujimoto et al., 1984). Similarly, in rabbits, chronic infusion of noradrenaline resulted in a significant decrease in β-AR density in tissues possessing predominantly β_1-ARs (heart and lung) but not in circulating lymphocytes which contain only β_2-ARs (Deighton et al., 1988). If, on the other hand, in the other forms of heart failure both noradrenaline and adrenaline are increased, there would be a concomitant down-regulation of both β_1- and β_2-ARs.

Another possible mechanism may be the presence of β_1-AR autoantibodies. Serum taken from patients with DCM, but *not* from patients with ICM or valvular disease, inhibits [^{3}H]DHA binding to rat cardiac membranes (predominantly β_1-ARs) but not to rat lung membranes (predominantly β_2-ARs) (Limas et al., 1989a; 1990a,b). Moreover, a serum γ-globulin fraction taken only from patients with DCM contained an autoantibody that was selectively directed against β_1-ARs (Wallukat and Wollenberger, 1987; Wallukat et al., 1990, 1991). However, this antibody had β-AR agonistic properties, because it produced an acceleration of beating in cultured neonatal rat heart cells without causing any desensitization (at least during a 48-h incubation), in sharp contrast to chronotropic responses to isoprenaline, which after 2 h of incubation were markedly reduced (Wallukat et al., 1990, 1991). And finally, the sera of 13 of 42 patients with DCM, but of *no* patients with ICM (of 17), monospecifically recognized an peptide corresponding to the sequence of the second extracellular loop of the human β_1-AR (Magnusson et al., 1990). Affinity chromatography-purified antibodies from these patients selectively inhibited ICYP binding to rat C6 glioma cell β_1-ARs. Also, in Chagas' disease antibodies against myocardial β_1- and spleen β_2-ARs have been detected (Sterin-Borda et al., 1988; Gorelik et al., 1990), but it is not known how cardiac β-ARs are changed in patients with Chagasic myocarditis. It would be of great interest to determine whether these antibodies affect cardiac β-ARs, which, in this case, should be a concomitant change in β_1- *and* β_2-ARs.

Because β-ARs in the human heart play an important physiological role in the regulation of cardiac function by mediating positive inotropic and chronotropic effects (Bristow et al., 1985; 1990), the decrease in β-AR function in chronic heart failure may contribute to the progression of the disease. Thus, one goal of future therapeutic interventions could be either to prevent β-AR down-regulation or to restore the impaired β-AR function. This possibly could be achieved either by decreasing sympathetic tone or by occupying β-ARs, thus preventing down-regulation.

Two such approaches are available at present: the use of either ACE inhibitors or β-AR antagonists. ACE inhibition may decrease sympathetic tone and, by this, restore the previously reduced cardiac β-AR density (see section VI.D). Thus, part of the beneficial effects of ACE inhibitors in the long-term treatment of patients with

chronic heart failure may be (despite their direct effects on the renin-angiotensin-aldosterone system) due to their inhibition of the elevated activity of the sympathetic nervous system.

β-AR antagonists could protect patients from the cardiotoxic effects of continuous exposure to elevated catecholamines (Schenk and Moss, 1966; Reichenbach and Benditt, 1970) by occupying β-ARs and preventing down-regulation of cardiac β-ARs (Fowler and Bristow, 1985; Shanes, 1987). Simultaneously, the use of these drugs could restore β-AR function (see section VI.B). However, as discussed, β_1- and β_2-ARs are differentially changed in different kinds of heart failure. Therefore, such differential changes should be considered before choosing either a β_1-AR-selective or a nonselective β-AR antagonist for treatment.

For example, in DCM with selectively down-regulated β_1-ARs but somewhat preserved β_2-ARs, a selective β_1-AR antagonist (e.g., metoprolol, atenolol, or bisoprolol) may be superior to a nonselective β-AR antagonist because β_1-AR-selective antagonists inhibit (and simultaneously up-regulate) only β_1-ARs but not β_2-ARs. Additional pulsatile treatment with a β_2-AR agonist could further improve cardiac performance especially if chronic β_1-AR antagonist treatment in ventricular myocardium would sensitize β_2-AR function (see section VI.B). In this context it is, however, important to note that the possible beneficial effects of such an additional treatment with β_2-AR agonists may quickly disappear, because recent studies in rats and guinea pigs showed that, during chronic isoprenaline infusion, cardiac β_2-ARs are significantly more rapidly and to a greater extent down-regulated than are cardiac β_1-ARs (Nanoff et al., 1989; Lu and Barnett, 1990; Molenaar et al., 1990).

In situations in which both β_1- and β_2-ARs are down-regulated (as in end-stage ICM or MVD), intermittent treatment with a nonselective β-AR antagonist *without* PAA should be effective, and β-AR agonists (or partial agonists) are likely to be harmful.

According to these considerations a better understanding of the actual β-AR status in patients with heart failure would be important for development of a more effective *individual* treatment regimen. Attempts have been made to follow β-AR changes in heart failure by measuring β-AR function in circulating lymphocytes which contain a homogeneous population of β_2-ARs (for references, see Motulsky and Insel, 1982; Brodde and Wang, 1988; Brodde et al., 1987, 1989a). Although the number of β-ARs in lymphocytes can correlate with that in solid tissues (Brodde et al. 1986a; Liggett et al., 1988, 1989b; Michel et al., 1989), the correlation between lymphocyte β_2- and cardiac β_1-ARs (which predominate in the human heart; cf. table 2) is much weaker than with cardiac β_2-ARs (Michel et al., 1986; Brodde et al., 1989a) and might completely disappear if local influences are involved.

For example, in patients chronically treated with different β-AR antagonists, the number of lymphocyte β_2-ARs was significantly correlated with the number of cardiac β_2-ARs but was not at all related to the number of cardiac β_1-ARs (Michel et al., 1988). Because circulating lymphocytes are composed of different subsets that differ in their β-AR density (Khan et al., 1986; Maisel et al., 1989a; Van Tits et al., 1990b) and the subset composition can be altered by a number of circumstances including rapid increases in endogenous catecholamines [i.e., during dynamic exercise (Landmann et al., 1988; Maisel et al., 1990a,b) or insulin-induced hypoglycemia (Frier et al., 1983; Van Tits et al., 1990a)] or exogenous administration of catecholamines (Crary et al., 1983; Van Tits et al., 1990b), this can result in difficulties in interpretation. A change in the subset composition of circulating lymphocytes has also been observed in patients with chronic heart failure (Maisel et al., 1990a,b); this could mimic or mask β-AR regulation and further complicate the interpretation of the lymphocyte-binding data.

A more precise method to establish cardiac β-AR status could be to determine the number of β-ARs in intracardiac biopsies; in fact, a good correlation between the number of β-ARs in ventricular endomyocardial biopsies and the number of β-ARs in whole ventricles does exist (Brodde et al., 1989a). Such an approach (when clinically indicated) could be helpful in determining the actual β-AR status in the failing heart and in improving the individual efficacy of treatment.

New solutions to the problem of desensitization of cardiac β-ARs following either agonist treatment or long-term exposure to elevated endogenous catecholamines may come from the rapidly growing knowledge on the mechanism of receptor desensitization. It has recently been shown that phosphorylation of the β-ARs by protein kinase A and/or β-AR kinase plays an important role in desensitization of the β-ARs. Thus, a very early step in the desensitization process seems to be an agonist-induced activation of protein kinase A (especially at low agonist concentrations) and an agonist-induced translocation of β-AR kinase from a cytosolic compartment to the cell plasma membrane (at high agonist concentrations). Both kinases phosphorylate agonist-occupied β-ARs to uncouple them from the G_s protein (for references, see Lefkowitz and Caron, 1986; Sibley and Lefkowitz, 1987; Hausdorff et al., 1990). Studies in which site-directed mutagenesis with the purified receptors was used demonstrate that it is possible to produce mutants that are protected against phosphorylation; in this case the magnitude of agonist desensitization was markedly reduced and the onset of desensitization was significantly delayed (Bouvier et al., 1988; Hausdorff et al., 1989; Liggett et al., 1989a).

The recent cloning of cDNA for β_1- (Yarden et al., 1986; Frielle et al., 1987), β_2- (Dixon et al., 1986; Kobilka

et al. 1987), and β_3-ARs (Emorine et al., 1989), $G_{s\alpha}$ protein (Kozasa et al., 1988), AC (Krupinski et al., 1989), and β-AR kinase (Benovic et al., 1989) opens intriguing possibilities for the precise study of the molecular mechanisms underlying agonist-induced desensitization. Such investigations could finally lead to therapeutic interventions in heart failure by drugs that will attenuate or prevent both the cardiac β-AR desensitization and downregulation that is induced by the disease-associated heightened sympathetic drive.

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