Vol. 121, No. 1, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 31, 1984 Pages 346-354

COMPARISON OF RAT AND HUMAN LEFT VENTRICLE BETA-ADRENERGIC RECEPTORS: SUBTYPE HETEROGENEITY DELINEATED BY DIRECT RADIOLIGAND BINDING

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Received April 4, 1984

Summary: Beta adrenergic receptors were identified in rat myocardial left ventricle and human papillary muscle by using the antagonist radioligand ^{3}H-dihydroalprenolol. The number (37.3 and 44.5 fmol/mg of protein, respectively in rat and man), and the K_D (1.6 and 2.8 nM, respectively in rat and man) of beta receptors were not significantly different.Adrenergic receptors of both beta 1 and beta 2 subtypes were found to coexist in the left ventricle. The relative proportions of the two beta receptor subtypes were determined by the use of competition radioligand selective binding and computer modelling techniques employing the subtype selective antagonists ICI 118,551 (beta 2 selective) and atenolol (beta 1 selective) in rat or metoprolol (beta 1 selective) in man.The rat left ventricle contained about 74% beta 1 and 26% beta 2 adrenergic receptors, human left ventricle papillary muscles contained about 69% beta 1 and 31% beta 2. Human and rat left ventricles contain both beta 1 and beta 2 adrenergic receptors with similar affinities. Rat might be a model for the study of human myocardial beta adrenergic receptors.

During the past several years, both in vivo and in vitro pharmacologic experiments have demonstrated the presence of beta adrenergic receptors in the myocardium (1-11). However many discrepancies still exist about the number and the affinity of these receptors in different animal species (1,2,4,9) and in different regions of the heart (3,6;7;8).Human heart receptors were predominantly studied in auricola and atrium (5,6), tissues both devoid of inotropism in vivo.

The studies performed on human left ventricles employed a high affinity radiojodinated ligand (3,7), which gives higher affinity values than those obtained by tritiated radioligand in rat tissue. Finally studies in which a tritiated antagonist was used were not aimed to investigate the subtype heterogeneity of left ventricle beta receptors (8,10,11).

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0006-291X/84 $1.50

In the present study we have used the same ligand (^{3}H-dihydroalprenolol) and the same experimental procedure to evaluate the binding characteristics and the affinities of rat and human left ventricles;further we have tried to delineate the subtype heterogeneity by direct radioligand binding in both species.

MATERIALS AND METHODS

Atenolol;ICI 118,551 and (+),(-),(±) propranolol were gifts from ICI-Pharma, ITALY.Metoprolol was a gift from Ciba-Geigy,Origgio,ITALY.All other chemicals used were purchased from the indicated sources: (-)-[^{3}H]-dihydroalprenolol ([^{3}H]-DHA)(specific activity,90-92 Ci/mmol)(New England Nuclear Corp.,Boston,MA); catecholamines (epinephrine and norepinephrine)(Sigma Chem. CO.,St.Louis,MO); EGTA,TRIS·HCl(Sigma Chem. CO.,St. Louis,MO).Other compounds were obtained from standard chemical suppliers.

Rat left ventricle

Male Sprague-Dawley rats weighing 200-250 g were killed by cervical dislocation and the hearts immediately (1-3 min) excised and placed in Krebs-Ringer buffer (4°C).Fat and great vessels were trimmed and removed.The hearts were placed in a shallow plastic dish filled with cold buffer and the atria separated from the ventricles by cutting along the atrioventricular sulcus.The interventricular septum and left ventricular free wall were then separated from the right ventricular free wall by grasping the septum with forceps and removing the right ventricular free wall as seen from above.The tissues were then finely minced and homogenized in cold buffer (30-35 volumes of 25 mM TRIS·HCl,pH 7.6;2 mM MgCl ; 1 mM EGTA;0.1 mM ascorbic acid) by using a Virtis tissue disruptor.Pooled tissues from four to seven rats were used for each experiment.The homogenate was centrifuged for 15 min at 120 x g and the supernate recentrifuged 3 times for 10 min at 39,000 x g to sediment the receptor membrane fraction.The final washed pellet was resuspended in the same buffer and used for protein determination (Bradford assay)(12) and for binding experiments.

Human left ventricles

Eight of 12 patients included in this study were receiving comparable preoperative medication of digoxin (0.250 mg/day) and furosemide (87±15 mg/day).None received antiarrhythmic drugs,anticoagulants or beta adrenergic blockers. None of the 12 patients received catecholamines at any time;4 patients were women.The mean ± SD age of all patients was 38±7 years (range,20-49 years). Average duration of cardiac symptoms was 20 months (range,18-30 months).The cardiac diagnosis was mitral valve stenosis (n=8) or mitral valve prolapse (n=4).In this study we did not employ tissues obtained from patients with associated aortic valve stenosis or insufficiency.All patients were in class I or II (New York Heart Association). The existence of coronary heart disease was excluded by pre-operative coronary angiography (13).

The papillary muscles(n=12) were placed in Krebs-Ringer buffer (4°C);connective tissue was trimmed and removed.The tissue was finely minced and homogenized in cold buffer (40 volumes of 25 mM TRIS HCl;pH 7.6; 2mM $MgCl_2$; 1 mM EGTA; 0.1 mM ascorbic acid) by using a Virtis tissue disruptor.Each papillary muscle was assayed independently.The subsequent procedures were the same as for rat tissue.

Binding assay

Binding of [^{3}H]-DHA to membranes was performed in polypropylene tubes which contained 0.1 to 10 n mol/l [^{3}H]-DHA;1 mg/ml membrane protein;with or without 100 μM (±)-propranolol.Total incubation volume was 200 μL.Duplicate reactions

were incubated for 30 min at 37°C and then terminated by addition of 3 ml ice cold 50 mM TRIS HCl,10 mM $MgCl_2$,pH 8.0,and vacuum filtered immediately on Whatman GF/B filters,which were then rinsed twice with 10 ml of the same buffer.Filters were dried,placed in a Triton X-100/toluene-based fluor,and radioactivity was determined in a Packard liquid scintillation spectrometer at an efficiency of 49%.

Competition experiments

Drugs competition with [^{3}H]-DHA for the membrane receptors was measured in tubes containing 1 nM [^{3}H]-DHA; 1 mg/ml membrane protein; 0.1 mM to 0.1 nM of the various drugs.Reactions were incubated,filtered and counted as for the binding experiments.

Data analysis

Specific binding was defined as the [^{3}H]-DHA binding displaceable by 0.1 mM (±)-propranolol both in Scatchard and competition experiments.Stereospecificity of the ligand binding to the membrane receptors was assessed by testing the displacement of bound [^{3}H]-DHA by (-)-propranolol and (+)-propranolol.The equilibrium dissociation constant (K_D) and the maximal number of binding sites (B_{max}) were calculated from plots according to Scatchard (14).Competition curves involving nonselective antagonists and selective ligands atenolol,metoprolol and ICI 118,551 were analyzed using a non linear least-squares fitting technique with statistical analysis,as recently published (15);this technique provides estimate and standard errors for the affinity constants of the competing ligand for each receptor subtype as well as proportions of each receptor subtype.The values of K_D,K_I and IC_{50} are expressed as mean ± SE;percentages of beta receptors are expressed only as mean and the range is given in parentheses.

RESULTS

Rat left ventricle

[^{3}H]-DHA bound to rat left ventricular membranes in a saturable manner as demonstrated in figure 1. The maximal binding capacity was 37.3 ± 5 fmol/mg (mean ± SE)(n=8) and the K_D was 1.6 ± 0.1 nM.Binding was stereospecific with (-)-propranolol (IC_{50} = 8 nM) more effective in displacing DHA than (+)-propranolol (IC_{50} = 500 nM)(figure 2).Table 1 shows the IC_{50} values of some adrenergic agonists. The potency series is consistent with beta adrenergic receptor subtype (16). To rigorously determine if beta 2 adrenergic receptors are present in membranes from left ventricle,we constructed competition curves with subtype selective ligands using atenolol (beta 1 selective) and ICI 118,551 (beta 2 selective) (17) and analyzed the data with computer modelling (15).

The relative proportion of beta 1 and beta 2 receptors is determined by assessing simultaneously the proportion of sites which bind ICI 118,551 with high affinity and atenolol with low affinity (beta 2 component) and similarly comparing the proportion of sites binding ICI 118,551 with low affinity and atenolol with high affinity (beta 1 component). The analysis then provides a single estimate of relative proportions based on all data. As shown in fig. 3 and 4, in membranes

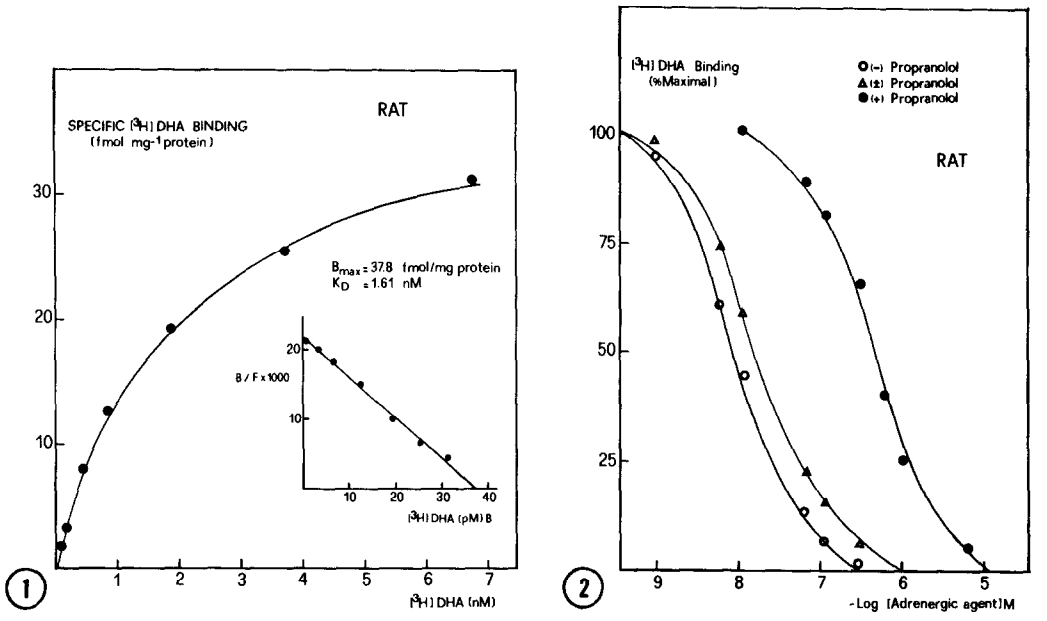


Figure 1 A typical single experiment of specific [^{3}H]-DHA binding to a membrane preparation of rat left ventricle (septum plus wall).The inset shows a Scatchard-plot.Mean K_D and B_{max} values were respectively 1.6 ±0.1 nM and 37.3±5 fmol/mg protein (mean of 8 experiments).

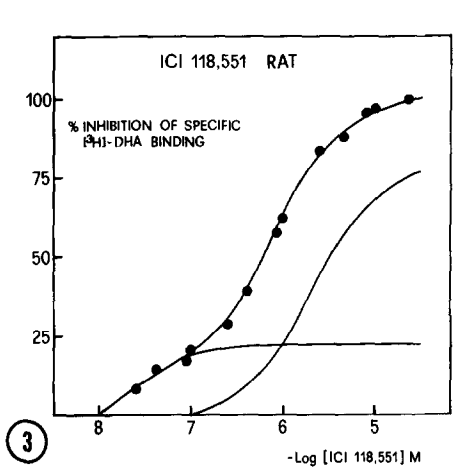
Figure 2 Competition curves for [^{3}H]-DHA binding to rat left ventricle membranes by(-),(±),(+)-propranolol.A representative experiment,replicated three times,is shown.

derived from left ventricle the competition curves are shallow and complex indicative of more than 1 subtype population. These data are more appropriately described by a 2 site than 1 site fit ($P<0.01$). This is contrast to the propranolol

Table 1 Potency series of various beta adrenergic drugs in competition with [^{3}H]-DHA in rat left ventricle and human papillary muscle membranes

	IC_{50} (M)	
Drugs	Rat	Man
Epinephrine	$8.0 \cdot 10^{-6}$ ± 1.0	$5.2 \cdot 10^{-6}$ ± 1.8
Norepinephrine	$4.0 \cdot 10^{-6}$ ± 1.2	$7.0 \cdot 10^{-6}$ ± 1.0
Isoproterenol	$5.5 \cdot 10^{-7}$ ± 0.9	$4.2 \cdot 10^{-7}$ ± 1.3
(-)-Propranolol	$8.0 \cdot 10^{-9}$ ± 1.5	$9.0 \cdot 10^{-9}$ ± 0.5
(±)-Propranolol	$1.2 \cdot 10^{-8}$ ± 0.2	$2.4 \cdot 10^{-8}$ ± 0.8
(+)-Propranolol	$5.0 \cdot 10^{-7}$ ± 1.9	$7.0 \cdot 10^{-7}$ ± 1.2

The [3H]-DHA sites demonstrate the potency series expected of beta adrenergic receptors,i.e. isoproterenol > epinephrine = norepinephrine. Stereospecificity is also indicated with (-)-propranolol > (+)-propranolol (see also figures 2 and 6).



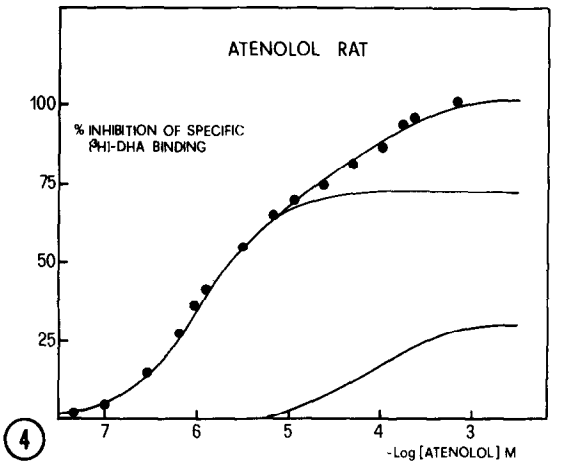


Figure 3 Competition curve by the specific beta adrenergic antagonist ICI 118,551 (beta 2 selective) for $[^3H]$-DHA binding to rat left ventricle membrane and its computer-assisted transformation into two one-site curves.A representative experiment,replicated four times,is shown.

Figure 4 Competition curve by the specific beta adrenergic antagonist atenolol (beta 1 selective) for $[^3H]$-DHA binding to rat left ventricle membranes and its computer-assisted transformation into two one-site curves.A representative experiment,replicated five times,is shown.

(nonselective) curve shown in figure 2 which is adequately modelled by a single homogeneous class of binding sites. This is to be expected since propranolol has equal affinity for beta 1 and beta 2 adrenergic receptors.The atenolol curve demonstrates (figure 4) a low affinity "foot" indicative of a small proportion of beta 2 adrenergic receptors, binding the drug with low affinity, and a high affinity "shoulder" indicative of a large proportion of beta 1 adrenergic receptors binding the drug with high affinity.

In contrast ICI 118,551 curves demonstrates a high affinity "shoulder" (figure 3) indicative of a small proportion of beta 2 adrenergic receptors binding the drug with high affinity and a low affinity (beta 2) "foot".

Thus both atenolol and ICI 118,551 distinguish the two subtypes of beta adrenergic receptors. Data derived from 4 curves (ICI 118,551) and 5 curves (atenolol) revealed that the percentage of beta 1 adrenergic receptors is 74% (range: 71-77%) and of beta 2 receptors is 26% (range: 23-29%). The higher affinity component of the atenolol curve (beta 1 component) had a K_I = 0.36 μM while the beta 2 component had a K_I = 16 μM. The ICI 118,551 curve demonstrated a high affinity component (beta 2) with a K_I = 8 nM and a low affinity beta 1 component with a K_I = 0.25 μM.

Human left ventricle

[^{3}H]-DHA bound to human papillary muscles in a saturable manner (figure 5). The maximal binding capacity was 44.5 ± 2 fmol/mg (n=6) and the K_D was 2.8 ± 0.3 nM (n=6). Binding was stereospecific with (-)-propranolol (IC_{50} = 9 nM) more effective in displacing DHA than (+)-propranolol (IC_{50} = 700 nM) (figure 6). Displacement experiments (figures 7 and 8), when analized as for rat tissues, demonstrated the presence of two subtypes of beta adrenergic receptors. Data derived from 7 curves (4 with ICI 118,551 and 3 with metoprolol) revealed that percentage of beta 1 adrenergic receptors is 69% (range 65-73%) and of beta 2 receptors is 31% (range 27-35%) (table 2). The higher affinity component of the metoprolol curve (beta 1 component) had a K_I = 0.57 µM and the low affinity component had a K_I = 52 µM. The ICI 118,551 curve demonstrated a high affinity component (beta 2) with a K_I = 5.7 nM and a low affinity component (beta 1) with a K_I = 1.7 µM.

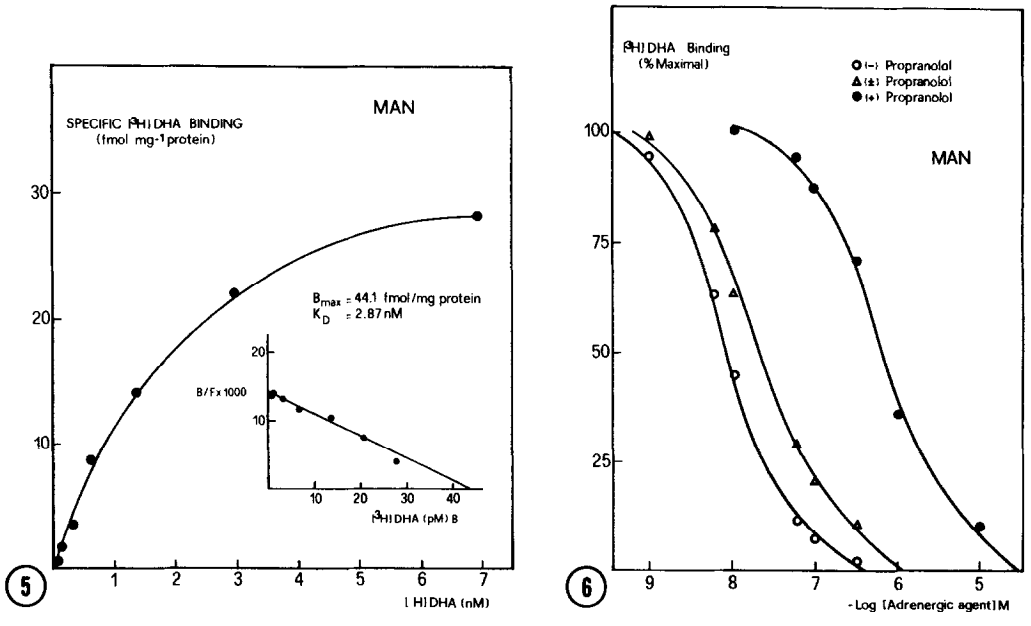


Figure 5 A typical single experiment of specific [^{3}H]-DHA binding to membranes of human papillary muscles. The inset shows a Scatchard-plot. Mean K_D and B_{max} values were respectively 2.8±0.3 nM and 44.5±2 fmol/mg protein (mean of 6 experiments).

Figure 6 Competition curves for [^{3}H]-DHA binding to human left ventricle papillary muscle membrane by (-), (±), and (+)-propranolol. A representative experiment, replicated two times, is shown.

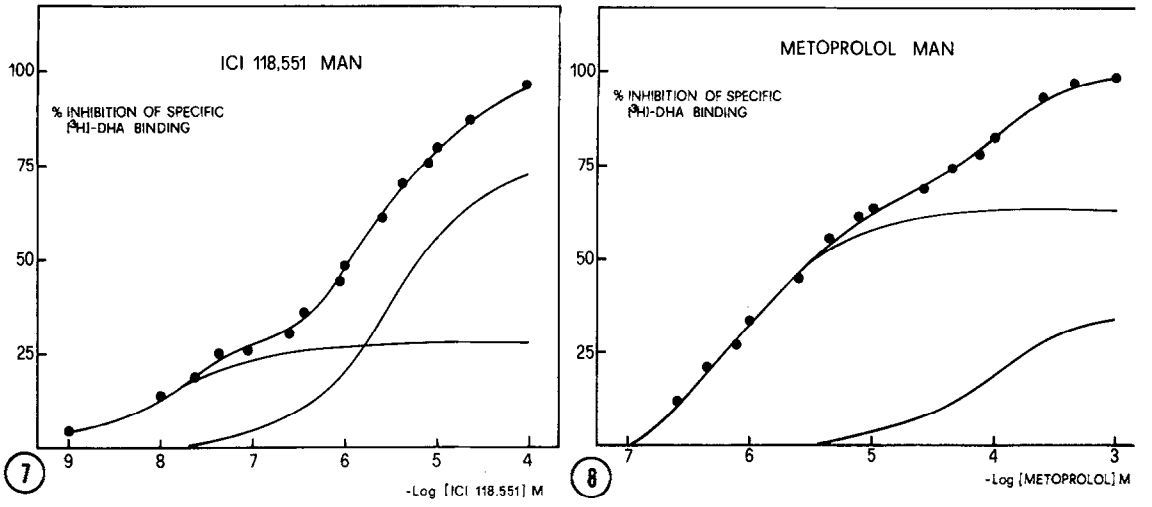


Figure 7 Competition curve for [^{3}H]-DHA binding to human left ventricle papillary muscle membranes by the specific beta adrenergic antagonist ICI 118,551 (beta 2 selective) and its transformation by a computer assisted technique into two one-site curves. A representative experiment, replicated four times, is shown.

Figure 8 Competition curve by the specific beta adrenergic antagonist metoprolol (beta 1 selective) for [^{3}H]-DHA binding to human left ventricle papillary muscle membranes and its transformation by a computer assisted technique into two one-site curves. A representative experiment, replicated three times, is shown.

DISCUSSION

The main findings of this work are the presence of beta 2 adrenergic receptors in rat and human left ventricle and the similarities between rat and man as regard to number, affinity and subclassification of beta adrenergic receptors. The demonstration of beta 2 adrenergic receptors in left ventricle is based on the analysis of inhibition curves of the radioligand by drugs which show in vitro

Table 2 Relative concentrations of beta 1 and beta 2 adrenergic receptors and K_I values in rat left ventricle and human papillary muscle membranes

Drugs	Subtypes % β_1/β_2	K_I (M) β_1	K_I (M) β_2
		RAT LEFT VENTRICLE	
Atenolol	71/29	$3.6 \cdot 10^{-7} \pm 0.4$	$1.6 \cdot 10^{-5} \pm 0.3$
ICI 118,551	77/23	$2.5 \cdot 10^{-7} \pm 0.3$	$8.0 \cdot 10^{-9} \pm 0.6$
		HUMAN PAPILLARY MUSCLE	
Metoprolol	65/35	$5.7 \cdot 10^{-7} \pm 0.3$	$5.2 \cdot 10^{-5} \pm 0.8$
ICI 118,551	73/27	$1.7 \cdot 10^{-6} \pm 0.5$	$5.7 \cdot 10^{-9} \pm 0.5$

different selectivity for beta 1 or beta 2 adrenoceptors;however several criteria must be fulfilled if such an analysis results in a valid in vitro assay for beta adrenergic receptor subtypes.Firstly the radioligand must bind to both adrenoceptor subtypes with the same affinity.This has been demonstrated for [^{3}H]-DHA in a variety of tissues where it labels both beta 1 and beta 2 adrenergic receptors with the same affinity (1,19),resulting in linear Scatchard plots and similar K_D values on all tissues studied so far (1,2,4,19).Secondly the interaction of each drug with the receptor should follow simple mass-action kinetics.Thus inhibition of binding by drugs which have the same affinity for both receptor subtypes should delineate a single class of receptors.This holds true for the present study;population of beta receptors,as evaluated by the computer assisted modelling technique in experiments employing propranolol,showed the "best fitting" with one class of beta receptors.Thirdly,on all tissues where both adrenergic receptor subtypes coexist,inhibition of binding by beta adrenergic selective drugs which have different affinities to beta 1 or beta 2 adrenergic receptors should result,at the same experimental conditions (20) in the delineation of the same percentages of beta adrenergic receptor subclasses.In this study this requirement is also fulfilled: ICI 118,551 (beta 2 selective)(17) and atenolol or metoprolol (beta 1 selective)(18) identified the same percentages of beta 1 and beta 2 receptors.It seems,therefore,to be justified to conclude that rat and human left ventricles contain a substantial amount of beta 2 adrenergic receptors.The presence of beta 2 receptors in isolated left ventricle of the rat has not been previously reported;studies have been usually performed on whole heart,including atria,which contains predominantly beta 2 receptors (1,2). In human beings the presence of beta 2 receptors in autopsied left ventricles has been recently reported (3,7),but the use of a radiojodinated ligand has precluded a direct comparison of the K_I with those obtained by tritiated ligands in rat.

The presence of a relevant amount of beta 2 adrenergic receptors in human and rat left ventricles support the notion that chronotropic and inotropic effects of adrenergic agonists may be mediated by different beta adrenergic receptors.The results of this study show some striking similarities between the characteristics and the number of beta adrenergic receptors in the left ventricle in human beings and in Sprague-Dawley rat.The reason why such a similarity was

not previously assessed is probably based on the use of whole heart in rat instead of isolated wall and septum (1,2,9),the use of jodinated ligands in human samples (3,7) and of tritiated ligands in rat (1,2,9),the use of human auricola instead of left ventricle (6).Among the species tested (9),thus,rat appears to be quite similar to human beings in the number,affinity and heterogeneity of the left ventricle beta adrenergic receptors.

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