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# Characterization and autoradiographic localization of β-adrenoceptor subtypes in human cardiac tissues

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**1** Receptor autoradiography using (−)-[$^{125}$I]-cyanopindolol (CYP) was used to study the distribution of β-adrenoceptor subtypes in human right atrial appendage, left atrial free wall, left ventricular papillary muscle and pericardium.

**2** The binding of (−)-[$^{125}$I]-CYP to slide-mounted tissue sections of human right atrial appendage was time-dependent ($K_1 = 4.11 \pm 1.01 \times 10^8$ M$^{-1}$ min$^{-1}$, $K_{-1} = 1.47 \pm 0.25 \times 10^{-3}$ min$^{-1}$, $n = 3$), saturable (42.02 ± 2.96 pM, $n = 4$) and stereoselective with respect to the optical isomers of propranolol (pKD (−):8.97 ± 0.02, (+):6.88 ± 0.06, $n = 3$).

**3** The proportions of β-adrenoceptor subtypes were determined in slide-mounted tissue sections using the antagonists CGP 20712A ($\beta_1$-selective) and ICI 118,551 ($\beta_2$-selective). In right atrial appendage and left ventricular papillary muscle 40% (34–45%) of the β-adrenoceptors were of the $\beta_2$-subtype.

**4** Images from X-ray film and nuclear emulsion coated coverslips exposed to (−)-[$^{125}$I]-CYP-labelled sections showed an even distribution of β-adrenoceptor subtypes over the myocardium of the right atrial appendage, left ventricular papillary muscle and left atrial free wall. Sections of pericardium exhibited predominantly $\beta_2$-adrenoceptors. $\beta_2$-Adrenoceptors were localized to the intimal surface of coronary arteries.

**5** The selective $\beta_1$-adrenoceptor agonist RO363 and $\beta_2$-selective agonist procaterol produced concentration-dependent inotropic responses in right atrial appendage strips. Responses to RO363 were antagonized by CGP 20712A ($pK_B = 9.29$) suggesting an interaction with $\beta_1$-adrenoceptors. Responses to procaterol were antagonized by ICI 118,551 ($pK_B = 9.06$) suggesting an interaction at $\beta_2$-adrenoceptors.

**6** The finding that a significant proportion of human myocardial adrenoceptors are of the $\beta_2$-subtype has important clinical implications for the involvement of these receptors in the control of heart rate and force, and the autoradiographic evidence suggests other roles in the coronary vasculature and pericardium.

## Introduction

The β-adrenoceptors which mediate positive inotropic and chronotropic responses in the heart were originally classified as $\beta_1$-adrenoceptors on the basis of functional studies (Lands *et al.*, 1967). This concept was a major stimulus for the development of clinically useful cardioselective β-adrenoceptor antagonists (Ablad *et al.*, 1973). In more recent studies using selective compounds, $\beta_2$-adrenoceptor-mediated positive inotropic and chronotropic responses have been detected in some (Carlsson *et al.*, 1972; Wagner *et al.*, 1981; Johansson & Persson, 1983; Kaumann *et al.*, 1983; Molenaar & Summers, 1987), but not all species (Juberg *et al.*, 1985). Homogenate radioligand binding studies have supported the existence of cardiac $\beta_2$-adrenoceptors in the atria of all species examined (for review see Stiles *et al.*, 1984).

In man, 20–50% of the β-adrenoceptors are of the $\beta_2$-subtype in right atrial appendage (Brodde *et al.*, 1983; Heitz *et al.*, 1983; Robberecht *et al.*, 1983; Stiles *et al.*, 1983; Golf *et al.*, 1985; Hedberg *et al.*, 1985) and 14–36% in left ventricle (Heitz *et al.*, 1983; Stiles *et al.*, 1983; Vago *et al.*, 1984; Golf *et al.*, 1985). In these studies utilizing homogenates the true myocardial $\beta_2$-adrenoceptor concentration is unknown because of

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the contribution of $\beta_2$-adrenoceptors from other cells such as fibroblasts (Lau *et al.*, 1980), nerves (Rand *et al.*, 1980; Lipe & Summers, 1986; Molenaar *et al.*, 1987) and on the endothelium of blood vessels (Lipe & Summers, 1986; Molenaar *et al.*, 1987; Stephenson & Summers, 1987). Functional studies carried out *in vivo* have also provided evidence for human cardiac $\beta_2$-adrenoceptors, since isoprenaline tachycardia is resistant to inhibition by $\beta_1$-selective adrenoceptor antagonists (Bonelli, 1978; Brown *et al.*, 1983; Arnold *et al.*, 1985). *In vitro*, $\beta_2$-adrenoceptor mediated inotropic responses have been found in both atrial and ventricular preparations (Ask *et al.*, 1985; Gille *et al.*, 1985; Mugge *et al.*, 1985; Zerkowski *et al.*, 1986).

The aim of the present study was to use autoradiographic techniques to localize $\beta_1$- and $\beta_2$-adrenoceptors in sections of human atrial and ventricular myocardium using the high affinity radioligand (−)-[$^{125}$I]-iodocyanopindolol ((−)-[$^{125}$I]-CYP) and the selective β-antagonists CGP 20712A ($\beta_1$; Dooley & Bittiger, 1984; Dooley *et al.*, 1986; Kaumann, 1986; Molenaar & Summers, 1987) and ICI 118,551 ($\beta_2$; Bilski *et al.*, 1980; 1983; O'Donnell & Wanstall, 1980). Functional responses to stimulation of these receptors have also been examined in the right atrial appendage by using the highly selective $\beta_1$-adrenoceptor agonist RO363 (McPherson *et al.*, 1984) and $\beta_2$-selective agonist procaterol (Yabuuchi, 1977).

## Methods

### *Preparation and sampling of tissues*

Tissue samples were obtained from patients undergoing cardiac surgery who had given informed consent to a protocol approved by the Epworth Hospital Ethics Committee. Samples were obtained from the right atrial appendage at the time of insertion of the right atrial cannula or from left ventricular papillary muscles in patients undergoing mitral valve replacement. The diagnoses and drug therapy of the patients studied are listed in Table 1. No patient received β-adrenoceptor antagonists during the two weeks before the operation. Tissues for autoradiographic processing were transported to the laboratory in a sealed flask at 4°C in a physiological salt solution (composition mM: NaCl 118.4, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 1.27, $Na_2PO_4$ 10.0, pH 7.4) containing Na heparin 50 iu ml$^{-1}$, whilst tissues for organ bath studies were transported in the physiological solution used for organ bath experiments (composition mM: NaCl 118.4, KCl 4.7, $CaCl_2$ 1.9, $NaHCO_3$ 25, $MgSO_4$ 1.2, glucose 11.7, $NaH_2PO_4$ 1.2, EDTA 0.1 and ascorbic acid 0.1, pH 7.4). Both solutions were equilibrated with 95% $O_2$ and 5% $CO_2$.

In the laboratory (15–20 min following surgical removal) the right atrial appendage, left atrial free wall and left ventricular papillary muscles were immersed in Krebs buffer diluted 1:1 with OCT embedding medium. Tissues were then frozen in isopentane previously cooled in liquid $N_2$. Sections (10 μm) were cut on a Reichert-Jung Cryostat at −20°C and mounted onto gelatin/chrome alum coated microscope slides (Young & Kuhar, 1979). Slide-mounted sections were pre-incubated with 0.1 mM guanosine triphosphate (GTP) in Krebs buffer containing 0.1 mM ascorbic acid and 10 μM phenylmethylsulphonylfluoride for 30 min, and then with the buffer and (−)-[$^{125}$I]-CYP (50–60 pM) at 25°C for 150 min with or without competing agent. Non-specific binding was determined by incubation with (−)-propranolol (1 μM).

Labelled sections were quickly rinsed in buffer followed by 2 × 15 min washes at 37°C in the same medium and finally a rinse in distilled water at 22–25°C. For biochemical assessment of atrial and left ventricular papillary muscle β-adrenoceptor binding sites, sections were wiped from the slides with Whatman GF/B filters and counted in a Packard gamma counter (Model 5301) at an efficiency of 79%. In autoradiographic studies, sections were dried in a stream of cold dehumidified air and stored at 4°C in sealed boxes containing silica gel until use.

### *Biochemical studies*

The association of (−)-[$^{125}$I]-CYP (50 pM) to specific binding sites in left and right atrial slide-mounted sections was determined at 0, 10, 20, 30, 40, 50, 60, 120 and 180 min. The dissociation of (−)-[$^{125}$I]-CYP from sections previously incubated with (−)-[$^{125}$I]-CYP for 150 min at 25°C was determined at 0, 15, 30, 45, 60, 75, 90, 120, 150, 180, 210, 240, 300, 360, 420 and 480 min after the addition of (−)-propranolol (1 μM).

Competition binding experiments were performed with the isomers of propranolol to examine stereoselectivity, and the $\beta_2$-selective antagonist ICI 118,551 (Bilski *et al.*, 1980; 1983; O'Donnell & Wanstall, 1980) and the $\beta_1$-selective antagonist CGP 20712A (Dooley & Bittiger, 1984; Dooley *et al.*, 1986; Kaumann, 1986; Molenaar & Summers, 1987) to determine the proportions of $\beta_1$- and $\beta_2$-adrenoceptor binding sites present. In competition binding experiments 20 concentrations of CGP 20712A (50 pM–0.5 mM) and 17 concentrations of ICI 118,551 (50 pM–20 μM) were used.

In kinetic experiments the observed association rate constant ($K_{obs}$) was given by the gradient of the linear plot (Tallarida & Murray, 1981) of log $(B_{eq}/B_{eq} - B_t)$ against time where $B_{eq}$ is the specific binding at equilibrium and $B_t$ is the binding at time $t$. The association rate constant, $K_1$, was then calculated from $K_1 = (K_{obs} - K_{-1})/(-)$-[$^{125}$I]-CYP (Williams & Lefk-

**Table 1** Details of patients from whom tissue samples were obtained for this study

| *Sex* | *Age* | *Diag* | *Tissue* | *Concurrent drug therapy* |
|---|---|---|---|---|
| | | | | Autoradiographic studies |
| F | 26 | WPW | RA | Flencainide |
| M | 18 | WPW | RA | Flencainide |
| M | 62 | IHD | RA | Isosorbide dinitrate |
| M | 67 | AVD | RA LA PE | Frusemide |
| F | 61 | MVD | PM | Frusemide, digoxin |
| M | 40 | MVD | PM | Digoxin |
| F | 36 | MVD | PM | Frusemide, digoxin |
| M | 60 | MVD | PM | Frusemide, digoxin |
| | | | | Organ bath studies |
| M | 76 | IHD | RA | Glyceryltrinitrate, digoxin, diltiazem |
| M | 42 | IHD | RA | Glyceryltrinitrate, frusemide, nifedipine, dipyridamole, aspirin, salbutamol, beclomethasone, diazepam, oxazepam, potassium chloride |
| M | 60 | IHD | RA | Glyceryltrinitrate, verapamil, dipyridamole, nitrazepam, trifluperazine |
| M | 42 | IHD | RA | Glyceryltrinitrate, verapamil, temazepam, paracetamol |
| F | 66 | IHD | RA | Glyceryltrinitrate, verapamil, prazosin |
| M | 51 | IHD | RA | Glyceryltrinitrate, nifedipine, dipyridamole, temazepam |

Diagnosis (diag): WPW, Wolf-Parkinson White Syndrome; IHD, ischaemic heart disease, AVD, aortic valve disease; MVD, mitral valve disease. Tissue samples; RA = right atrial appendage, LA = left atrial free wall, PM = left ventricular papillary muscle, PE = pericardium. Patients routinely received papaveretum 15–20 mg and hyoscine hydrobromide 0.3–0.4 mg 1 h prior to preparation for anaesthesia. Anaesthesia was induced with diazepam, phenoperidine or fentanyl and 66% $N_2O$ in oxygen. Patients were paralysed with pancuronium and anaesthesia was maintained with further doses of phenoperidine or fentanyl. Blood pressure was controlled with glyceryltrinitrate.

owitz, 1978). The dissociation rate constant $K_{-1}$, was determined by polyexponential curve fitting (Brown & Manno, 1978) of the decrease in binding following the addition of 1 μM (−)-propranolol. Saturation and competition binding data were analysed by two computer programs, EBDA (McPherson, 1983) which performed preliminary Scatchard, Hill & Hofstee analyses and created a file for the iterative curve fitting programme LIGAND (Munson & Rodbard, 1980) used to obtain final parameter estimates. Statistical analysis was performed using the $F$ ratio test to measure the goodness of fit of the competition binding curves, for either one or two sites.

*X-ray film and nuclear emulsion autoradiography*

Right atrial appendage, left atrial free wall, left ventricular papillary muscle and pericardium sections were incubated for 150 min with (−)-[$^{125}$I]-CYP in the absence or presence of ICI 118,551 (70 nM) to block selectively $\beta_2$-adrenoceptors or CGP 20712A (100 nM) to block selectively $\beta_1$-adrenoceptors or (−)-propranolol (1 μM) to define non-specific binding. X-ray film (Fuji RX) was apposed to dried labelled sections in light tight boxes for 7 days (30 days for the pericardial sections). The film was developed with Kodak D19, briefly rinsed in water and fixed with Kodak Rapid Fix. Emulsion coated coverslips (Kodak NTB3) were exposed to the same slide-mounted sections for 2 days then developed in Kodak Dektol, briefly rinsed in water and fixed in Kodak Rapid Fix at the paper dilution. Sections were stained with pyronine Y, dried and mounted for light microscopy. Every tenth section was set aside and stained with haematoxylin and eosin for histological examination.

*Organ bath studies*

Right atrial appendage preparations were dissected at 4°C into 4 strips (< 1 mm thickness) and then mounted under 0.5 g tension in physiological saline aerated with 95% $O_2$ and 5% $CO_2$ and maintained at 37°C. Myocardial strips were driven at a frequency of 1.0 Hz with square wave pulses of 5 ms duration at 1.5 times the threshold voltage using a Grass SD9 stimulator. Neuronal and extraneuronal uptake mechanisms and α-adrenoceptors were inhibited with phenoxybenzamine (50 μM, 30 min incubation, followed by 6 washes over 30 min). The tissues were then exposed to a sensitizing concentration (0.1 μM) of (−)-isopren-

aline. Following washout (8 washes in 60 min) one cumulative concentration-response curve to (−)-isoprenaline was established. Of two myocardial strips, one was exposed to the $\beta_2$-adrenoceptor selective antagonist ICI 118,551 (Bilski *et al.*, 1980; 1983; O'Donnell & Wanstall, 1980) and the other to the $\beta_1$-selective antagonist CGP 20712A (Dooley & Bittiger, 1984; Dooley *et al.*, 1986; Kaumann, 1986; Molenaar & Summers, 1987) and allowed to incubate for 60 min. Cumulative concentration-response curves to the $\beta_2$-adrenoceptor selective agonist procaterol (Yabuuchi, 1977) or $\beta_1$-selective agonist RO363 (McPherson *et al.*, 1984) were then obtained in all 4 strips.

Changes in isometric tension were recorded on a Grass model 7c polygraph using a Grass FTO3c transducer. Responses to each concentration of agonist were expressed as the change in tension from basal values. Calculations were made in each individual experiment of $pD_2$ values (for half-maximal responses of agonist) and the intrinsic activity ($\alpha$, (−)-isoprenaline = 1). The degree of antagonism produced by each antagonist was assessed by calculating the mean dose-ratio (DR) of the agonist in the absence and presence of the antagonist. The $pK_B$ value of the antagonist was then calculated from the equation $pK_B = -\log (DR - 1) - \log[B]$ (Furchgott, 1972) where DR = dose-ratio of agonist and [B] = concentration of antagonist.

Results are expressed as mean ± s.e.mean.

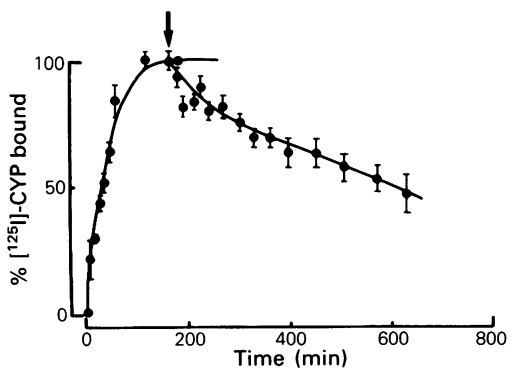


**Figure 1** Kinetic analysis of (−)-[$^{125}$I]-cyanopindolol (CYP) binding to human atrial sections. Association curve represents specific binding of (−)-[$^{125}$I]-CYP (50–60 pM) at 25°C against time. Dissociation curve represents specific binding at various times after addition of 1 μM (−)-propranolol (arrow) to sections incubated with (−)-[$^{125}$I]-CYP for 150 min. Each point is expressed as a percentage of the binding occurring at equilibrium in association experiments and at time zero in dissociation experiments. Vertical lines indicate the s.e.mean from three separate experiments carried out in duplicate.

*Drugs*

The drugs used were: (−)- and (+)-propranolol; ICI 118,551 (erythro-DL-1(7-methylindan-4-yloxy)-3-isopropylamino-butan-2-ol) (Imperial Chemical Industries); CGP 20712A (2-hydroxy-5(2-((2-hydroxy-3-(4-((1-methyl-4-trifuoromethyl)1H-imidazole-2-yl)-phenoxy)propyl)amino) ethoxy)-benzamide monomethane sulphonate) (Ciba-Geigy); (−)-isoprenaline bitartrate (Wyeth); RO363 ((−)-1-(3,4-dimethoxyphenethylamino)-3-(3,4-dihydroxyphenoxy) -2-propanol)oxalate) (gift from Dr E. Malta); procaterol hydrochloride (Warner Lambert); Na$^{125}$I (Amersham International); (−)-cyanopindolol (Sandoz, Basle); (−)-[$^{125}$I]-CYP was prepared from (−)-CYP and Na$^{125}$I as previously described (Lew & Summers, 1985); guanosine triphosphate (Boehringer Mannheim); NTB3 nuclear emulsion, Rapid Fix, D19,

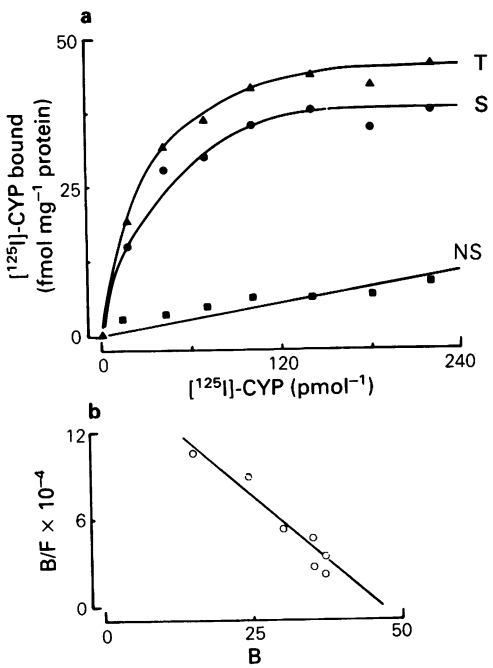


**Figure 2** Equilibrium binding analysis of specific (−)-[$^{125}$I]-cyanopindolol (CYP) binding to slide-mounted sections of human atria. Sections were incubated for 150 min with (−)-[$^{125}$I]-CYP (10–200 pM) at 25°C. (a) Binding curves which are representative of four similar experiments conducted in triplicate, showing total (T), specific (S) and non-specific (NS) binding defined by 1 μM (−)-propranolol. (b) Shows the corresponding Scatchard plot of bound (fmol mg$^{-1}$ protein) against bound/free ligand. The iterative curve fitting program LIGAND (Munson & Rodbard, 1980) was used to determine the values for $B_{max}$ (46.4 fmol mg$^{-1}$ protein) and $K_D$ (33.6 pM).

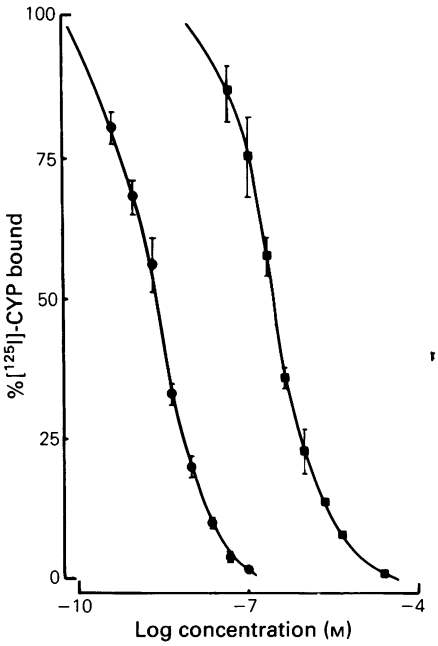


**Figure 3** Stereoselective inhibition of (−)-[$^{125}$I]-cyanopindolol (CYP) binding (50–60 pM) to slide-mounted sections of right atrial appendage by the (−)-(●) and (+)-(■) isomers of propranolol. The (−)-isomer was 123 times more potent than the (+)-isomer at competing for (−)-[$^{125}$I]-CYP binding sites. Points show mean from 3 experiments conducted in duplicate; vertical lines indicate s.e.mean.

Dektol (Kodak); pyronine Y, haematoxylin (Sigma); eosin (Medos).

Stock solutions (10 mM) of CGP 20712A and ICI 118,551 were prepared in 0.01 M HCl and the remaining drugs in distilled water. Dilutions were made using the appropriate Krebs buffer containing 1 mM ascorbic acid. All other chemicals were of analytical grade.

## Results

### *Biochemical characterization of (−)-[$^{125}$I]-CYP binding to slide-mounted human atrial and left ventricular papillary muscle sections*

Before autoradiography was carried out it was necessary to ensure that (−)-[$^{125}$I]-CYP was bound to sites with the characteristics of β-adrenoceptors in sections of human myocardium. In kinetic experiments the association of (−)-[$^{125}$I]-CYP to binding sites in slide-mounted atrial sections at 25°C was time-dependent and reached equilibrium at 120 min (Figure 1). The

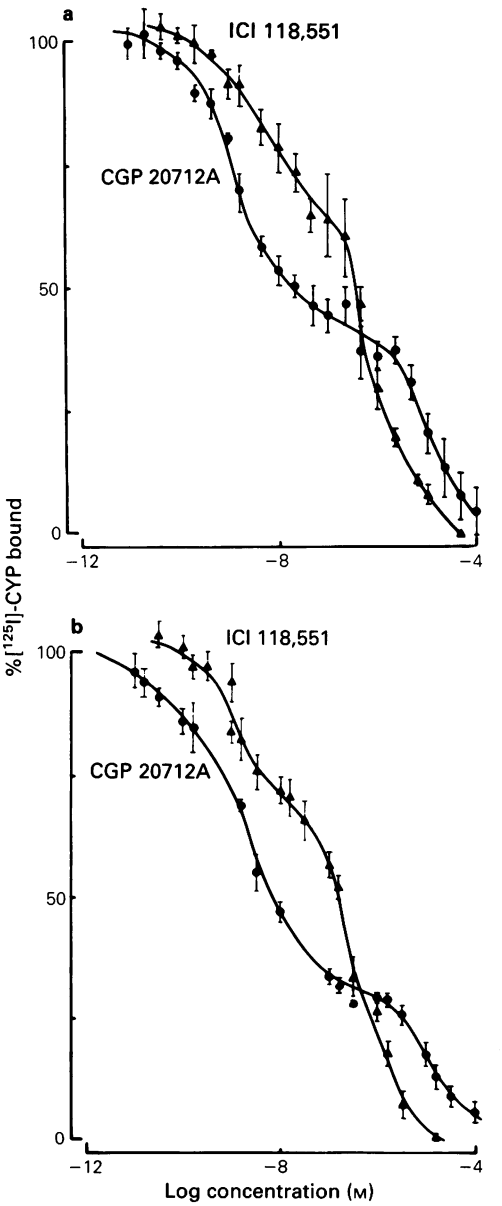


**Figure 4** Inhibition of specific (−)-[$^{125}$I]-cyanopindolol (CYP) binding with increasing concentrations of the $\beta_1$-adrenoceptor antagonist, CGP 20712A (●) or $\beta_2$-adrenoceptor antagonist, ICI 118,551 (▲) in slide-mounted sections of (a) right atrial appendage and (b) left ventricular papillary muscle. The binding curves were biphasic and characterized by low pseudo Hill coefficients. Two binding sites corresponding to $\beta_1$- and $\beta_2$-adrenoceptors were present in the ratios of 60% (55–66%) and 40% (34–45%), respectively. Points show mean for 3 experiments conducted in duplicate; vertical lines indicate s.e.mean.

observed association rate constant ($K_{obs}$) determined from the gradient of the linear plot of bound ligand as a function of time (see Methods) by linear regression (Tallarida & Murray, 1981) was $0.023 \pm 0.005\ min^{-1}$ ($n = 3$). The dissociation of (−)-[$^{125}$I]-CYP from binding sites occurred monoexponentially consistent with binding to a single site (Figure 1). The dissociation rate constant determined from the polyexponential curve fitting program (Brown & Manno, 1978) was $1.47 \pm 0.25 \times 10^{-3}\ min^{-1}$ ($n = 3$). The association rate constant $K_1$ was found to be $4.11 \pm 1.01 \times 10^8\ M^{-1}\ min^{-1}$ ($n = 3$).

The specific binding of (−)-[$^{125}$I]-CYP to slide-mounted sections was saturable. Total, specific and non-specific binding and a Scatchard transformation of specific binding are shown for a representative experiment in Figure 2. The mean dissociation constant and maximal number of binding sites derived from non-linear least squares fitting of the data using LIGAND and a single site model (Munson & Rodbard, 1980; McPherson, 1983) were $42.02 \pm 2.96$ pM ($n = 4$) and $46.4 \pm 12.4\ fmol\ mg^{-1}$ protein, respectively.

The binding of (−)-[$^{125}$I]-CYP was stereoselective for the isomers of propranolol. Competition curves obtained with the (−)- and (+)-isomers, analysed using LIGAND and a single site model, gave $pK_D$ values of $8.97 \pm 0.02$ and $6.88 \pm 0.06$ ($n = 3$) (Figure 3). Thus the specific binding of (−)-[$^{125}$I]-CYP to slide-mounted atrial sections showed the characteristics of binding to β-adrenoceptors.

*Quantitative analysis of the proportions of $\beta_1$- and $\beta_2$-adrenoceptor subtypes in human atrial and left ventricular papillary muscle sections*

Competition experiments using the highly selective $\beta_1$-antagonist CGP 20712A (Dooley & Bittiger, 1984; Dooley *et al.*, 1986; Kaumann, 1986; Molenaar & Summers, 1987) and selective $\beta_2$-adrenoceptor antagonist ICI 118,551 (Bilski *et al.*, 1980; 1983; O'Donnell & Wanstall, 1980) produced shallow biphasic competition curves in both atria and left ventricular papillary muscle (Figure 4). These curves could be resolved into 2 components ($P < 0.001$ for a 2 site fit using the $F$-ratio test) using LIGAND (Munson & Rodbard, 1980). The $pK_D$ values for CGP 20712A at high affinity $\beta_1$- and low affinity $\beta_2$-sites were similar in atria ($\beta_1$, $9.20 \pm 0.03$; $\beta_2$, $5.56 \pm 0.01$; $n = 3$), and left ventricular papillary muscle ($\beta_1$, $9.10 \pm 0.04$; $\beta_2$, $5.41 \pm 0.05$; $n = 4$). The $pK_D$ values for ICI 118,551 were also similar in atria ($\beta_1$, $6.41 \pm 0.14$; $\beta_2$, $9.17 \pm 0.44$; $n = 5$) and left ventricular papillary muscle ($\beta_1$, $6.39 \pm 0.11$; $\beta_2$, $8.90 \pm 0.26$; $n = 5$) (Table 2). The proportion of $\beta_2$-adrenoceptors in the right atrial appendage was 37–45% and in the left ventricular papillary muscle 34–37%.

*Autoradiographic localization of β-adrenoceptors in atria, left ventricular papillary muscle and pericardium*

Autoradiographic localization of the β-adrenoceptor subtypes was examined in sections of right atrial appendage ($n = 4$), left ventricular papillary muscle ($n = 3$), left atrial free wall ($n = 1$) and pericardium ($n = 1$). X-ray film images of (−)-[$^{125}$I]-CYP binding to right atrial appendage, left atrial free wall (data not shown) and left ventricular papillary muscle showed an even distribution of both $\beta_1$- and $\beta_2$-adrenoceptor subtypes at all levels of the tissue examined (Figure 5). X-ray images of sections of the pericardium revealed an almost homogeneous population of $\beta_2$-adrenoceptors since binding was almost completely inhibited by ICI 118,551. The density of these sites was lower than in the myocardium.

High resolution studies with nuclear emulsion coated coverslips confirmed the distribution of $\beta_2$-adrenoceptors found in the X-ray film images, with $\beta_2$-adrenoceptors localized over cardiac muscle cells (Figure 6). In the left ventricular papillary muscles a

**Table 2** Competition binding curves for (−)-[$^{125}$I]-cyanopindolol and the $\beta_1$-selective antagonist CGP 20712A or the $\beta_2$-selective antagonist ICI 118,551

| | *nH* | $pK_D\beta_1$ | $pK_D\beta_2$ | $\%\beta_1$ | $\%\beta_2$ |
|---|---|---|---|---|---|
| | *Right atrial appendage* | | | | |
| CGP 20712A | 0.34 ± 0.01 | 9.20 ± 0.03 | 5.56 ± 0.01 | 54.6 ± 2.6 | 45.4 ± 2.6 |
| ICI 118,551 | 0.66 ± 0.09 | 6.41 ± 0.14 | 9.17 ± 0.44 | 62.8 ± 5.7 | 37.2 ± 5.7 |
| | *Left ventricular papillary muscle* | | | | |
| CGP 20712A | 0.36 ± 0.01 | 9.10 ± 0.04 | 5.41 ± 0.05 | 65.5 ± 2.0 | 34.5 ± 2.0 |
| ICI 118,551 | 0.56 ± 0.05 | 6.39 ± 0.11 | 8.90 ± 0.26 | 62.6 ± 4.7 | 37.4 ± 4.7 |

Shown are pseudo Hill coefficients (nH), dissociation constants at $\beta_1$- and $\beta_2$-adrenoceptor binding sites ($pK_D\beta_1$, $pK_D\beta_2$) and their proportions ($\%\beta_1$, $\%\beta_2$) in slide-mounted 10 μm sections of human right atrial appendage and left ventricular papillary muscle. Data are mean ± s.e.mean from 3–5 separate determinations conducted in duplicate.

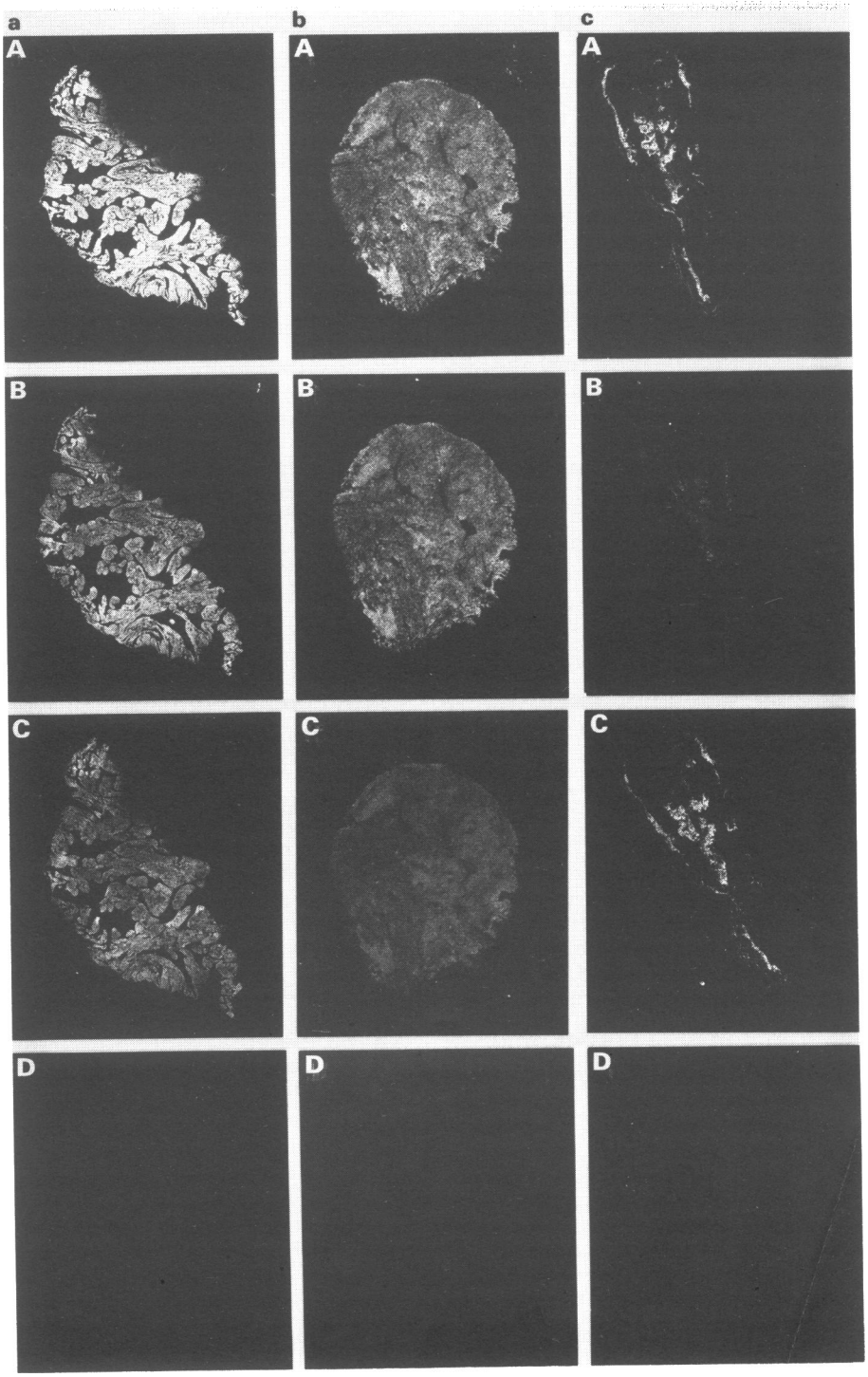


**Figure 5** Photographs of X-ray film images showing the distribution of (−)-[$^{125}$I]-cyanopindolol (CYP) binding sites in serial sections of (a) right atrial appendage, (b) left ventricular papillary muscle and (c) pericardium. Sections were incubated with 50 pM (−)-[$^{125}$I]-CYP in the absence (A) or presence of (B) 70 nM ICI 118,551, to occlude $\beta_2$-adrenoceptors, or (C) 100 nM CGP 20712A, to occlude $\beta_1$-adrenoceptors, or (D) 1 μM (−)-propranolol, to occlude both $\beta_1$- and $\beta_2$-adrenoceptors. Note the high and even distribution of $\beta_1$- and $\beta_2$-adrenoceptor sites in the right atrial appendage and left ventricular papillary muscle. The pericardium showed a minor population of $\beta_1$- and predominance of $\beta_2$-adrenoceptors.

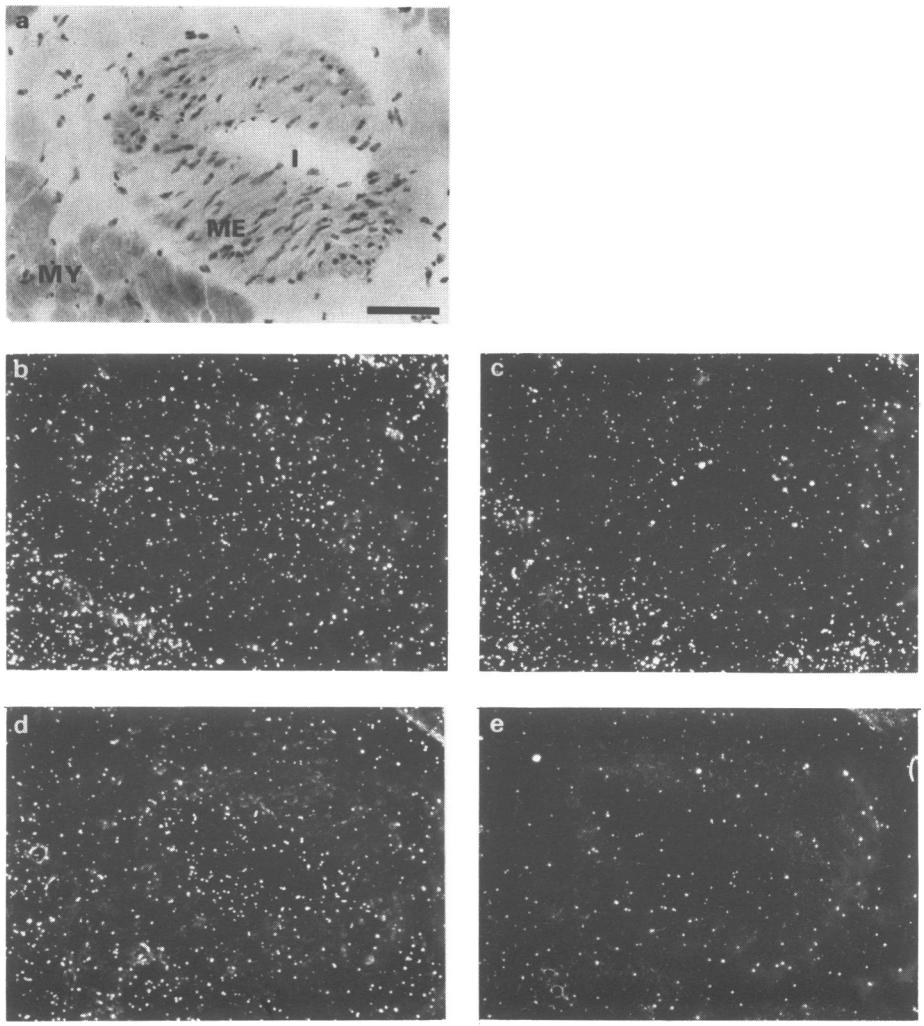


**Figure 6** Light microscopic autoradiographic localization of (−)-[$^{125}$I]-cyanopindolol (CYP) binding to serial sections of left ventricular papillary muscle. (a) Shows a photomicrograph of a haematoxylin and eosin stained section of coronary artery showing media (ME), intima (I) and surrounding myocardium (MY). (b–e) Darkfield micrographs of sections incubated with (−)-[$^{125}$I]-CYP in the absence (b) or presence of (c) 70 nM ICI 118,551 or (d) 100 nM CGP 20712A or (e) 1 μM (−)-propranolol and apposed to nuclear emulsion coated coverslips. Note the high density of evenly distributed $\beta_1$- (c) and $\beta_2$- (d) adrenoceptor binding sites over the myocardium and the absence of binding over the media of the coronary artery. There was a low density of β-adrenoceptor sites over the intimal surface of the coronary artery which were of the $\beta_2$-subtype (b and d). The scale bar represents 50 μm.

small amount of $\beta_2$-adrenoceptor binding was found over the intimal surface of small intramyocardial blood vessels. The density of vascular $\beta_2$-adrenoceptor binding sites in this area was much lower than that of myocardial $\beta_2$-adrenoceptors.

*Organ bath studies*

(−)-Isoprenaline, the highly selective $\beta_1$-adrenoceptor agonist RO363 and the $\beta_2$-selective agonist procaterol produced positive inotropic responses in electrically

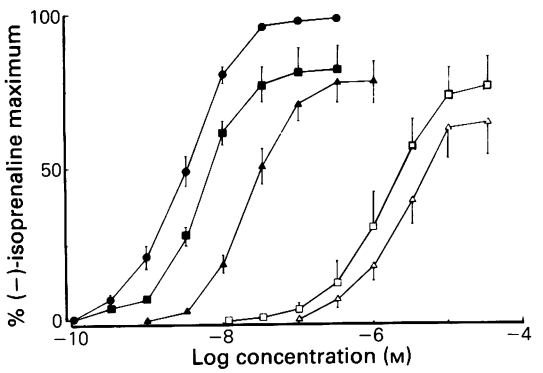


**Figure 7** Mean cumulative concentration-response curves for (−)-isoprenaline (●), RO363, in the absence (■) and presence (□) of CGP 20712A (100 nM), and procaterol, in the absence (▲) and presence (△) of ICI 118,551 (70 nM), in electrically driven human right atrial appendage strips. Inotropic responses are expressed as a percentage of the maximum response to (−)-isoprenaline. Points shown are mean values from 4–22 experiments; vertical lines indicate s.e.mean.

driven human right atrial appendage strips. Figure 7 shows mean cumulative concentration-response curves to the three agonists. RO363 and procaterol were nearly full agonists in this preparation and produced their responses through activation of $\beta_1$- and $\beta_2$-adrenoceptors, respectively. Table 3 shows mean intrinsic activity values and $PD_2$ values for half-maximal responses. The selective $\beta_1$-adrenoceptor antagonist CGP 20712A (100 nM) produced a 195 fold shift of the RO363 concentration-response curve. The $pK_B$ value for CGP 20712A against RO363 was 9.29, a value which is similar to its known affinity at the $\beta_1$-adrenoceptor (Dooley & Bittiger, 1984; Dooley *et al.* 1986; Kaumann, 1986; Molenaar & Summers, 1987). The selective $\beta_2$-adrenoceptor antagonist ICI 118,551 (70 nM) produced an 83 fold shift of the procaterol concentration-response curve. The $pK_B$ value for ICI 118,551 against procaterol was 9.06, a value which is in accordance with its affinity at the $\beta_2$-adrenoceptor (McPherson *et al.*, 1984). The $pK_B$ values for CGP 20712A and ICI 118,551 are also closely similar to $pK_D$ values determined at $\beta_1$- and $\beta_2$-adrenoceptors, respectively, in biochemical experiments in the right atrial appendage and left ventricular papillary muscle in this study. These studies show that at least a proportion of the $\beta_1$- and $\beta_2$-adrenoceptors on the myocardium shown in the autoradiographs mediate positive inotropic responses.

## Discussion

The specific binding of (−)-[$^{125}$I]-CYP to atrial sections displayed the binding characteristics appropriate for β-adrenoceptors (McPherson *et al.*, 1984). The affinity of the ligand for β-adrenoceptors in the slide-mounted sections was similar to that found in homogenate binding studies of human atria (Brodde *et al.*, 1983; Heitz *et al.*, 1983; Robberecht *et al.*, 1983; Stiles *et al.*, 1983; Golf *et al.*, 1985; Hedberg *et al.*, 1985) and cardiac β-adrenoceptors of other animal species (McPherson *et al.*, 1984). The receptor density ($B_{max}$) was also similar in sections and in some homogenate binding studies of human atria (Robberecht *et al.*, 1983; Stiles *et al.*, 1983; Golf *et al.*, 1985; Hedberg *et al.*, 1985), yet half that found in other studies (Brodde *et al.*, 1983; Hedberg *et al.*, 1985).

The percentage of $\beta_2$-adrenoceptors in atrial sections was higher than that in some studies (20–26%) (Brodde *et al.*, 1983; Heitz *et al.*, 1983; Stiles *et al.*, 1983; Golf *et al.*, 1985) but less than that in others (50–55%) (Robberecht *et al.*, 1983; Hedberg *et al.*, 1985). Procedures which are known to affect the β-adrenoceptor density in binding studies include drug treatment (Hedberg *et al.*, 1985), prolonged exposure (> 6 h) to room temperature (Stiles *et al.*, 1983), and the use of racemic ligands (Burgisser *et al.*, 1981). In this study drug treatments known to affect β-adrenoceptor number (Hedberg *et al.*, 1985) were avoided

**Table 3** $pD_2$ values and intrinsic activities (α, (−)-isoprenaline = 1) for the positive inotropic effects, in strips of human right atrial appendage, of (−)-isoprenaline, RO363 and procaterol

| | $pD_2$ | α | n |
|---|---|---|---|
| (−)-Isoprenaline | 8.48 ± 0.07 | 1.00 | 22 |
| RO363 | 8.28 ± 0.10 | 0.83 ± 0.08 | 4 |
| RO363 + 100 nM CGP 20712A | 6.01 ± 0.13 | 0.78 ± 0.10 | 4 |
| Procaterol | 7.62 ± 0.07 | 0.79 ± 0.07 | 10 |
| Procaterol + 70 nM ICI 118,551 | 5.67 ± 0.11 | 0.66 ± 0.11 | 4 |

Values shown are mean ± s.e.mean from *n* experiments.
$PD_2$ value = − log $EC_{50}$ for half-maximal responses. The effects of RO363 and procaterol were evaluated in the absence and presence of 100 nM CGP 20712A and 70 nM ICI 118,551, respectively.

by careful selection of patients. Samples were frozen rapidly after removal (20 min) to prevent temperature-induced changes and (−)-[$^{125}$I]-CYP was used as the radioligand.

Selective down regulation of $\beta_1$-adrenoceptors secondary to chronic elevation of catecholamine levels in plasma (Snavely *et al.*, 1982) may also reduce the proportion of $\beta_1$-adrenoceptors in patients with cardiac failure (Bristow *et al.*, 1982; 1986; Cohn *et al.*, 1984). In this study atrial tissue was taken from patients in which heart failure was absent and papillary muscle from patients with mild heart failure.

In homogenate binding studies $\beta_2$-adrenoceptors derived from cardiac fibroblasts (Lau *et al.*, 1980), nerves (Rand *et al.*, 1980; Lipe & Summers, 1986; Molenaar *et al.*, 1987) and blood vessels (Lipe & Summers, 1986; Molenaar *et al.*, 1987; Stephenson & Summers, 1987) may increase the apparent proportions of $\beta_2$-adrenoceptors assigned to cardiac muscle cells. The autoradiographic studies indicate that this is not the case in the human tissues used here, since $\beta_2$-adrenoceptors were found primarily on cardiac muscle cells in both atria and left ventricular papillary muscle. Only a small contribution would be made from vascular $\beta_2$-adrenoceptors and none from the pericardium which was studied separately from the myocardial tissues.

The similar proportion of $\beta_2$-adrenoceptors found in left ventricular papillary muscle and artria further support the concept of $\beta_2$-adrenoceptors associated with the myocardium rather than sympathetic nerve terminals, since there is little sympathetic innervation of the human ventricle (Chidsey & Braunwald, 1966). Dahlof *et al.* (1982) have also shown no change in $\beta_2$-adrenoceptor number in cat atria following destruction of the sympathetic nerve terminals with 6-hydroxydopamine. The differences in innervation and the similar proportions of $\beta_1$- and $\beta_2$-adrenoceptors in atria and left ventricular papillary muscle argue against the concept that $\beta_1$-adrenoceptors respond primarily to neuronally released noradrenaline and $\beta_2$-adrenoceptors to circulating catecholamines (Zaagsma *et al.*, 1979; Bryan *et al.*, 1981; Ariens & Simonis, 1983).

This study using agonists and antagonists with the highest reported selectivity to date shows clearly delineated $\beta_1$- and $\beta_2$-adrenoceptor-mediated positive inotropic responses in the human right atrial appendage. The high potency of the three $\beta$-adrenoceptor agonists used here indicates a receptor reserve in this preparation. This supports results from other studies using human atrial and left ventricular muscle preparations (Gille *et al.*, 1985; Mugge *et al.*, 1985; Zerkowski *et al.*, 1986; Ask *et al.*, 1985; Bristow *et al.*, 1986).

The demonstration of cardiac $\beta_2$-adrenoceptors also has implications for the pathophysiology and treatment of heart failure, where selective down regulation of $\beta_1$-adrenoceptors associated with high sympathetic drive (Bristow *et al.*, 1982; 1986; Cohn *et al.*, 1984) may increase the contribution of $\beta_2$-adrenoceptors to the positive inotropic and chronotropic response. A pool of cardiac $\beta_2$-adrenoceptors which mediate inotropic responses and which are resistant to down regulation (Bristow *et al.*, 1986) highlights the therapeutic potential of $\beta_2$-agonists in the treatment of heart failure.

Results from *in vivo* functional studies have also provided evidence for $\beta_2$-adrenoceptor-mediated control of human heart rate. Isoprenaline tachychardia is resistant to $\beta_1$-adrenoceptor blockade in man (Bonelli, 1978; Brown *et al.*, 1983; Arnold *et al.*, 1985) and chronotropic responses mediated by $\beta_2$-adrenoceptors have been directly documented in animal studies (Kaumann, 1986). Thus there is a potential role for $\beta_2$-adrenoceptors in the pathophysiology of arrhythmias. Additionally, functional studies of positive inotropic and chronotropic responses, may have underestimated the role of cardiac $\beta_2$-adrenoceptors controlling other cellular functions such as cellular potassium levels (Clausen & Flatman, 1980), gluconeogenesis and glycogenolysis (Hems & Whitton, 1980; Weiner & Taylor, 1985).

In conclusion, autoradiographic studies provide a direct demonstration of myocardial $\beta_2$-adrenoceptors in human atria and left ventricular papillary muscle. The potential clinical and therapeutic importance of these cardiac $\beta_2$-adrenoceptors has yet to be fully realized.

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