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Relationship between β-adrenoceptors and calcium channels in human ventricular myocardium

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1 The stoichiometric relationship between adrenoceptors and saturable binding sites for 1,4-dihydropyridines in calcium channels was investigated in human ventricular myocardium. Membrane particles were prepared from heart specimens of patients undergoing open heart surgery. The patients suffered from hypertrophic obstructive cardiomyopathy (HOCM) or mitral valve disease.

2 Using [^{3}H]-prazosin and [^{125}I]-2-β-hydroxy-3-iodiphenyl-ethyl-aminoethyl tetralone ([^{125}I]-HEAT) as labels we detected only a marginal density of α_1-adrenoceptors, regardless of disease. No α_2-adrenoceptors were detected with [^{3}H]-rauwolscine.

3 In HOCM patients we estimated 72 ± 10 fmol mg^{-1} ($n = 12$) β-adrenoceptors labelled with [^{3}H]-(−)-dihydroalprenolol and 74 ± 5 fmol mg^{-1} ($n = 2$) β-adrenoceptors labelled with [^{125}I]-(−)-iodocyanopindolol; the equilibrium dissociation constants K_D, were 1.2 ± 0.2 nmol l^{-1} for [^{3}H]-(−)-dihydroalprenolol and 7 ± 1 pmol l^{-1} for [^{125}I]-(−)-iodocyanopindolol. In patients with mitral valve disease we estimated 84 ± 11 fmol mg^{-1} ($n = 3$) labelled with [^{3}H]-(−)-dihydroalprenolol and 66 ± 13 fmol mg^{-1} ($n = 2$) labelled with [^{125}I]-(−)-iodocyanopindolol. The K_D values were 1.8 ± 0.6 nmol l^{-1} for [^{3}H]-(−)-dihydroalprenolol and 8 ± 2 pmol l^{-1} for [^{125}I]-(−)-iodocyanopindolol.

4 In 14 HOCM patients we estimated 107 ± 12 fmol mg^{-1} calcium channel sites labelled with [^{3}H]-nimodipine with a K_D of 280 ± 4 pmol l^{-1}. In 5 patients with mitral valve disease the density of calcium channel sites labelled with [^{3}H]-nimodipine was 78 ± 5 fmol mg^{-1} with a K_D of 290 ± 20 pmol l^{-1}, In HOCM patients the density of calcium channel sites labelled with the benzoxadiazol 1, 4-dihydropyridine ([^{3}H]-(+)-PN 200–110) was 1.6 fold of that labelled with [^{3}H]-nimodipine with a K_D of 84 ± 11 pmol l^{-1}.

5 In a group of 4 HOCM patients in which calcium channels were labelled with [^{125}I]-iodipine, the density of sites was 1.37 ± 0.07 fold the density of sites labelled by [^{3}H]-(+)-PN 200-11-. The K_D value of [^{125}I]-iodipine was 246 ± 16 pmol^{-1}. (+)-PN 200–110 was approximately 100 fold more potent than (−)-PN 200–110 as a competitor of [^{125}I]-iodipine binding.

6 For the HOCM group a significant correlation was found between β-adrenoceptor density and calcium channel density, whereas in the mitral valve group no such correlation was found. This does not prove that there is causal interaction leading to a relationship between the density of β-adrenoceptors and calcium channels. However, because positive inotropic effects of catecholamines mediated by β-adrenoceptors are associated with opening of calcium channels, this suggests that the density of both β-adrenoceptors and calcium channels could be co-regulated.

Introduction

Contractile strength of isolated preparations of human heart is enhanced by catecholamines through an interaction with β-adrenoceptors (Harms, 1976; Kaumann *et al.*, 1982; Gille *et al.*, 1985). When β-adrenoceptors are saturated with a specific β-adrenoceptor antagonist, α-adrenoceptor agonists have been reported to cause inconsistent positive inotropic effects which are blocked by α-adrenoceptor antagonists in human atrium (Skomedal *et al.*, 1985) and ventricle (Bruckner *et al.*, 1984).

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Both β-adrenoceptors (Reuter & Scholz, 1977) and α-adrenoceptors (Bruckner & Scholz, 1984) can mediate an increase of slow inward calcium current caused by catecholamines in mammalian heart muscle. The positive inotropic effects of catecholamines appear related in a complex manner to an augmented slow inward calcium current to a major degree (β-adrenoceptor; Beeler & Reuter, 1970) or to some degree (α-adrenoceptor; Bruckner & Scholz, 1984). The increase of slow inward calcium current mediated by either β- or α-adrenoceptors appears to be related to phosphorylation of a 15-KDa protein of the sarcolemma (Lindemann, 1986).

What is the stoichiometry of calcium channels to adrenoceptors? To answer this question, we labelled both putative calcium channels and adrenoceptors in membrane particles prepared from the ventricle of individual patients. Fresh ventricular tissues were made available to us from patients with mitral valve lesion or hypertrophic obstructive cardiomyopathy (HOCM). We chose conventional radioligands for α_1, α_2-and β-adrenoceptors and radiolabelled 1,4-dihydropyridines as radioligands for saturable binding sites within or on putative calcium channels. Because the estimate of saturable binding sites can be a function of the ligand used (Glossmann *et al.*, 1985) we occasionally labelled the calcium channels of a single patient with 3 different dihydropyridines. Our data provide information on the proportion of putative calcium channels (labelled by high-affinity ligands) to β-adrenoceptors.

Methods

Patient material

Portions of left ventricular papillary muscle were excised from 5 patients of either sex suffering from combined mitral valve lesion. These patients underwent replacement of the mitral valve. Fourteen additional patients of either sex had hypertrophic obstructive cardiomyopathy (HOCM). The patients with HOCM underwent partial ablation of ventricular septal tissue. None of the 19 patients received a β-adrenoceptor blocking agent for one week before the operation. Anaesthesia was with enfluorane (ethrane) with fentanyl for induction and pancuronium for muscle relaxation. The excised valve and adhering papillary muscle or ventricular septal tissues were quickly transported on ice to the laboratory in a solution as described previously (Kaumann *et al.*, 1982). The solution contained (mmol l^{-1}): Na^+ 140, K^+5, Ca^{2+} 2.25, Mg^{2+}0.5, Cl^-98.5, HCO_3^-34, HPO_4^{2-}1, SO_4^{2-}0.5, fumarate 5, pyruvate 5, L-glutamate 5, glucose 10, disodium ethylenediamine tetraacetic acid (EDTA) 0.04, equilibrated with 95% O_2 and 5% CO_2. The water was deionized and twice distilled in glass.

Membrane particles

Human ventricular tissues were transported in the oxygenated physiological solution and dissected on ice. Membrane particles were prepared as described by Kaumann & Birnbaumer (1974). Fractions of the whole homogenate and the 1000 *g* pellet were also kept for subcellular fractionation studies. All membrane fractions were stored at −80°C and later under liquid nitrogen. Protein was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard.

Binding assays

Binding was usually carried out at 37°C, except the assays with α-adrenoceptor ligands which were at 25°C. Binding of [^{3}H]-nimodipine (Ferry *et al.*, 1985a), [^{3}H]-(±)-Bay K 8644 (Janis *et al.*, 1984) and [^{3}H]-(+)-PN 200–110 (Goll *et al.*, 1983) was performed in 50 mmol l^{-1} Tris HCl, 0.1 mmol l^{-1} phenylmethysulphonyl-fluoride and 1 mmol l^{-1} ascorbic acid at pH 7.4, using 1 μmol l^{-1} unlabelled nimodipine to define blank binding in a volume of 0.25 ml allowing 30 min for equilibrium to be reached. The assay was then diluted with 3.5 ml of ice-cold wash buffer, and bound and free radioactivity separated through Whatman GF/C filters. For [^{3}H]-nimodipine the range of ligand employed in saturation isotherms was 100–2500 pmol l^{-1} and for [^{3}H]-(+)-PN 200–110 it was 40–4000 pmol l^{-1}.

[^{125}I]-iodipine binding was performed in a volume of 0.25 ml for studies of protein-dependency and binding inhibition employing 50–60 pmol l^{-1} of radioligand. For [^{125}I]-iodipine saturation isotherms, [^{125}I]-iodipine in ethanol was pipetted into 1 ml Eppendorf tubes with a single polypropylene pipette tip going from the lowest to the highest quantity of radioactivity. The ethanol was lyophylised away and buffer added. The [^{125}I]-iodipine in buffer was pipetted into assay tubes using a single pipette tip going from the lowest (10 pmol l^{-1}) to the highest concentration (750 pmol l^{-1}). All additions were made to a total assay volume of 0.15 ml, and the assay tubes individually counted; blanks were defined by 1 μmol l^{-1} (±)-PN 200–110. Following 1 h at 25°C the tubes were incubated at 37°C for 30 min before bound and free ligand was separated. Binding inhibition studies were performed as for saturation studies in 0.25 ml allowing 30 min before separation of bound and free ligand. Nine concentrations of drugs were employed.

Binding of [^{3}H]-(−)-dihydroalprenolol (0.1 to 5 nmol l^{-1}) was measured as for [^{3}H]-nimodipine, but in a buffer of 50 mmol l^{-1} Tris HC1, 1 mmol l^{-1} EDTA

pH 7.4 with blanks defined by 1 μmol l^{-1} (−)-alprenolol.

Saturation binding of [^{125}I]-(−)-iodocyanopindolol (10–500 pmol l^{-1}) was measured as for [^{3}H]-(−)-dihydroalprenolol except that 2 h were allowed when bound and free ligand were separated.

Binding of [^{3}H]-prazosin (Hornung *et al.*, 1979), was measured as for [^{3}H]-nimodipine binding but in 50 mmol l^{-1} Tris HCl, 1 mmol l^{-1} EDTA, allowing 30 min for equilibrium to be reached at 25°C. Blanks were defined by 10 μmol l^{-1} phentolamine. In the experiments of Table 1 in which 1.9 nmol l^{-1} [^{3}H]-prazosin was used, true receptor binding was calculated by subtracting the phentolamine displaceable filter binding.

Binding of [^{3}H]-rauwolscine (Perry & U'Prichard, 1981) was measured as for [^{3}H]-prazosin. In the experiment with 4.5 nmol l^{-1} [^{3}H]-rauwolscine shown in Table 1, receptor binding was corrected by subtracting the phentolamine displaceable filter binding. In one HOCM patient, we used the α_1-adrenoceptor-selective drug [^{125}I]-2-β-4-hydroxy-3-iodiphenyl-ethyl-aminomethyl tetralone ([^{125}I]-HEAT) (Glossmann *et al.*, 1981), allowing 1 h to separate bound and free ligand. Incubation was in the medium described for [^{3}H]-prazosin and [^{3}H]-rauwolscine.

In the experiments shown in Table 1, binding assays with [^{3}H]-(−)-dihydroalprenolol and [^{3}H]-(+)-PN 200–110 were also performed at 25°C.

Analysis of data

Saturation isotherms were fitted with a non-linear regression programme for a single class of homogeneous non-interacting binding sites (De Lean *et al.*, 1978). Binding inhibition data were fitted by non-linear regression to the general dose-response equation (De Lean *et al.*, 1978). For racemic ligands only the bindable enantiomer was taken into account. For [^{125}I]-iodipine saturation analysis the radioactivity in each tube was counted when all assay additions had been made. After filtration the counts for each blank were correlated against the total ligand added and the slope of the blank. For each total binding tube the blank was calculated and subtracted to yield specific binding. This procedure takes account of the 5–10% variation in tracer between assay tubes. Statistical analysis of differences between means was performed with Student's two tailed *t* test.

Drugs

[^{3}H]-nimodipine (145–150 Ci $mmol^{-1}$, radiochemical purity 99%), unlabelled nimodipine and [^{3}H]-(±) Bay K 8644 (80 Ci $mmol^{-1}$) and unlabelled (±)-Bay K 8644 were from Bayer AG (Wuppertal, F.R.G.). [^{3}H]-(+)-PN 200–110 (isopropyl-4-(2, 1, 3-benzoxadiazol-4-yl) 1,4-dihydro-2, 6-dimethyl-5-methoxy-carbonyl-pyrridine-3-carboxylate) (75 Ci $mmol^{-1}$) was from Amersham (U.K.). [^{125}I]-iodipine (2200 Ci $mmol^{-1}$) was synthesized as described before by Ferry & Glossmann (1984). Unlabelled enantiomers of PN 200–110 and 202 791 (isopropyl 4-benzoxadiatol-4-yl-,4-dihydro-3, 6-dimethyl-5-nitro-3-pyridine carboxylate) were a gift from Sandoz (Basle, Switzerland). (±)-CGP 28, 392 (4-(2 difluornormethoxyphenyl)-1, 4, 5, 7-tetrahydro-2-methyl-5-oxofurol (3, 4) pyridine-3-carboxylic acid ethylester) was a gift from CIBA-Geigy, (Basle Switzerland). The 1, 4-dihydropyridines used were dissolved in dimethylsulphoxide (DMSO) at 10 mmol l^{-1} and stored at −20°C in the absence of u.v. light. [^{3}H]-(−)-dihydroalprenolol (102 Ci $mmol^{-1}$) and [^{3}H]-rauwolscine (87 Ci $mmol^{-1}$) were from NEN (Dreieich, F.R.G.). [^{125}I]-(−)-iodocyanopindolol was synthesized in our laboratory as described by Hoyer *et al.* (1982). [^{3}H]-prazosin (33 Ci $mmol^{-1}$ was from Amersham U.K. [^{125}I]-HEAT (2200 Ci $mmol^{-1}$) was prepared as described by Glossmann *et al.* (1981). Phentolamine methanesulphonate was from CIBA-Geigy (Basle, Switzerland). (−)-Alprenolol hydrochloride was from Hassle AB (Molndal, Sweden).

Table 1 Specific binding sites for ligands in human ventricular membranes (fmol mg^{-1})

Patient	*([^{3}H]-prazosin* (1.9 nmol l^{-1})	*[^{3}H]-rauwolscine* (4.5 nmol l^{-1})	*[^{3}H](−)-dihydroalprenolol* (1.3 nmol l^{-1})	*[^{3}H]-(+)-PN 200–110* (3.0 nmol l^{-1})
HOCM 29.11.83	16	5	44	139
HOCM 15.06.84	11	0	36	143
HOCM 08.05.84	3	0	39	129
Mitral 22.06.83	24	0	74	155
Mitral 20.04.82	0	4	78	193
$\bar{x}$ ± s.d.	11 ± 10	2 ± 2	54 ± 20	152 ± 25

The binding assays were carried out at 25°C.
Specific binding was calculated from fmol g^{-1} = (total d.p.m.) − (blank d.p.m. + filter blank d.p.m.) × (d.p.m. $fmol^{-1}$) × (mg^{-1}).

Results

Characteristics of the membrane preparation

Specific binding sites were purified ($n = 3$) 1.20 ± 0.27 fold for [^{3}H-]-(−)-dihydroalprenolol (1.0–1.5 nmol l^{-1}) and 1.38 ± 0.07 fold for [^{3}H]-nimodipine (0.3–0.5 nmol l^{-1}) in the 1000 *g* membrane pellet with respect to the whole homogenate. Purification was 1.46 ± 0.12 fold for [^{3}H]-(−)-dihydroalprenolol and 1.44 ± 0.17 fold for [^{3}H]-nimodipine in the 11,000 *g* pellet. These data suggest that removal of soluble proteins in the supernatant did not influence differentially the specific binding of the two ligands. Therefore we conclude that we did not create an artificial stoichiometry of β-adrenoceptors to calcium channels with the method of membrane isolation (Kaumann & Birnbaumer, 1974) used.

Marginal presence of α-adrenoceptors

In membranes from 3 HOCM patients with 2 patients with mitral valve disease we carried out in parallel binding of the α_1-selective antagonist [^{3}H]-prazosin, α_2-selective antagonist [^{3}H]-rauwolscine, β-adrenoceptor ligand [^{3}H]-(−)-dihydroalprenolol and putative calcium channel ligand [^{3}H]-(+)-PN 200–110. Assays were performed at 25°C in order to allow comparison with the results of α-adrenoceptor binding previously reported (Perry & U'Prichard, 1981) and because non-specific binding for the α-adrenoceptor ligands appeared lower than at 37°C. Table 1 shows that only insignificant specific binding was detected for the α-adrenoceptor ligands. Furthermore, in one experiment using 10–1000 pmol l^{-1} [^{125}I]-HEAT on membranes of an HOCM patient no specific binding was detected (not shown). On the other hand, tissues from all patients exhibited significant β-adrenoceptors and putative calcium channels.

Differential labelling of calcium channels by [^{3}H]-nimodipine, [^{3}H]-(+)PN 200–110 and [^{125}I]-iodipine

At the beginning of this study (April, 1982) [^{3}H]-nimodipine was the 1,4-dihydropyridine which had been employed to label calcium channels in guinea-pig brain (Ferry & Glossman, 1982), and rat heart (Janis *et al.*, 1982). During the course of this study the other ligands became available. When sufficient membranes were prepared from an individual patient calcium channels were labelled with as many probes as possible.

[^{3}H]-(±)-Bay K 8644 did not bind specifically in the concentration range 0.15 to 5.3 nmol l^{-1} to human heart membranes.

Saturation isotherms with [^{3}H]-nimodipine are shown in Figure 1. [^{3}H]-nimodipine labelled significantly more sites in the HOCM group (107 ± 12 fmol mg^{-1}) than in the mitral lesion group, (78 ± 5 fmol mg^{-1}) (Table 2). In 8 HOCM patients saturation analysis was performed with the benzoxadiazol 1,4-dihydropridine [^{3}H]-(+)-PN 200–110 and [^{3}H]-nimodipine. An experiment is shown in Figure 1. [^{3}H]-(+)-PN 200–110 labelled on average 1.60 ± 0.07 fold as many sites (168 ± 29 fmol mg^{-1}) as [^{3}H]-nimodipine (Table 2).

Binding of [^{3}H]-(+)-PN 200–110 was inhibited stereoselectively by the enantiomers of PN 200–110, the (+)-enantiomer being 260 fold more potent than the (−)-enantiomer (Table 3). The binding of [^{3}H]-(+)-PN 200–110 was inhibited by both calcium channel activators (±)-Bay K 8644 (Schramm *et al.*, 1983) and (+)-(S)202–791 (Kongsamut *et al.*, 1985, Hoff *et al.*, 1985)], and blockers (+)-PN 200–110 and (−)-(**R**) 202–791 (Kongsamut *et al.*, 1985, Hof *et al.*, 1985) (Figure 2).

In four HOCM patients calcium channels were labelled with [^{125}I]-iodipine, [^{3}H]-(+)-PN 200–110 and [^{3}H]-nimodipine (Table 4). Although there was

Table 2 Summary of saturation data

Ligand	B_{max} *(fmol mg^{-1}) values* HOCM	Mitral	K_D *values (nmol l^{-1})* HOCM	Mitral
[^{3}H]-nimodipine	107 ± 12 ($n = 14$)	78 ± 5 ($n = 5$)	0.28 ± 0.04 ($n = 14$)	0.29 ± 0.02 ($n = 5$)
[^{3}H]-(+)-PN 200–110	168 ± 29 ($n = 8$)	—	0.08 ± 0.01 ($n = 8$)	—
[^{125}I]-iodipine	161 ± 17 ($n = 5$)	—	0.25 ± 0.05 ($n = 4$)	—
[^{3}H]-(−)-dihydroalprenolol	72 ± 10 ($n = 12$)	84 ± 11 ($n = 5$)	1.2 ± 0.2 ($n = 12$)	1.8 ± 0.6 ($n = 5$)
[^{125}I]-(−)-iodocyanopindolol	74 ± 5 ($n = 2$)	66 ± 13 ($n = 2$)	0.007 ± 0.001 ($n = 2$)	0.008 ± 0.002 ($n = 2$)

Data are arithmetic means ± s.e.mean.

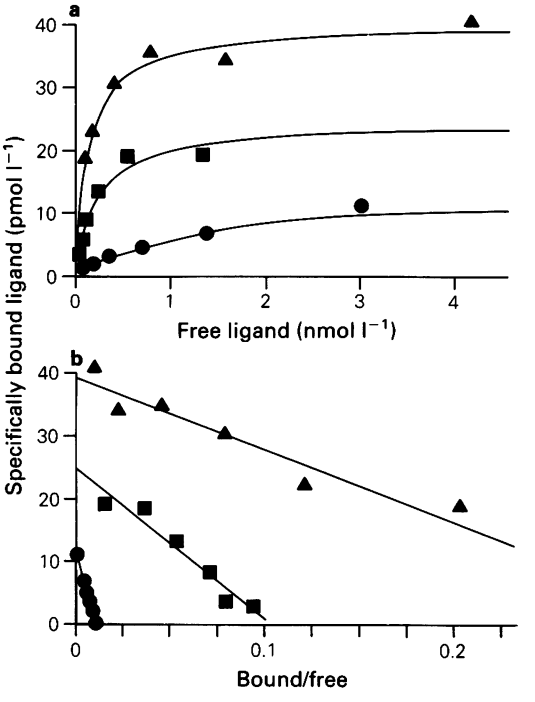

Figure 1 Saturation analysis with [^{3}H]-(+)-PN 200–110 (▲), [^{3}H]-nimodipine (■) and [^{3}H]-(−)-dihydroalprenolol (●) in ventricular membranes derived from an HOCM patient (19.5.82). Blanks as defined in Methods have been subtracted and specific binding is plotted in (a). In (b), Hofstee plots of the data in panel (a) are shown. The *Ki* and B_{max} values are: [^{3}H]-(+)-PN 200–110, 108 pmol l^{-1} and 182 fmol l^{-1}; [^{3}H]-nimodipine, 280 pmol l^{-1} and 115 fmol l^{-1}; [^{3}H]-(−)-dihydroalprenolol, 1.10 nmol l^{-1} and 58 fmol mg^{-1}. All three saturation experiments were performed on the same day in parallel employing 35 μg of membrane protein per assay tube.

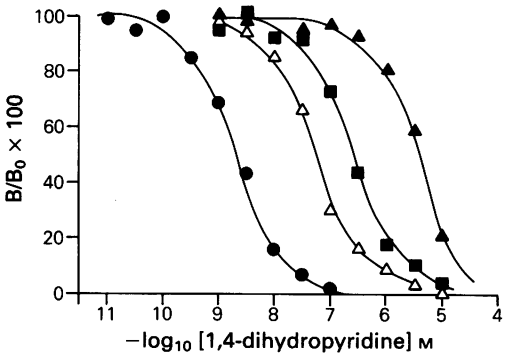

Figure 2 Pharmacological profile of calcium channels labelled with [^{3}H]-(+)-P 200–110: 620 pmol l^{-1} was used with 50 μg of membrane protein. Total binding was 34 pmol l^{-1} and blanks 4.8 pmol l^{-1}. B_0 is specifically bound [^{3}H]-(+)-PN 200 110 and B specific in the presence of indicated added drug. the *Ki* and nH values for the competing drugs are (+)-PN 200–110 (●), 310 pmol l^{-1} and 0.96; (−)-R 202–791 (△), 8.8 nmol l^{-1} and 0.91; (+)-S-202–791 (▲) 450 nmol l^{-1} and 1.1, (±)-Bay K 8644 (■), 42 nmol l^{-1} and 0.97.

variability among the four patients, the rank order of B_{max} was consistently [^{125}I]-iodipine> [^{3}H]-PN 200–110 > [^{3}H]-nimodipine in each patient. [^{125}I]-iodipine bound to 2.28 ± 0.21 fold as many sites as [^{3}H]-nimodipine. Binding characteristics of [^{125}I]-iodipine are shown in Figure 3. Binding was linear with protein concentrations up to 0.1 mg ml^{-1}. High affinity binding of [^{125}I]-iodipine was inhibited by the enantiomers of PN 200–110 with a 120 fold stereoselectivity (Table 3).

β-Adrenoceptors correlate with putative calcium channels in HOCM patients

A saturation isotherm of the β-adrenoceptor radioligand [^{3}H]-(−)-dihydroalprenolol is shown in Figure 1. Mean B_{max} values and K_D values for [^{3}H]-(−)-dihydroalprenolol and [^{125}]-(−)-iodocyanopindolol are shown in Table 2. The density of saturable binding sites for [^{3}H]-nimodipine correlated significantly with the density of β-adrenoceptors (labelled with either [^{3}H]-(−)-dihydroalprenolol or [^{125}I]-(−)-iodocyanopindolol) in 14 HOCM patients but not in five patients with mitral lesion (Figure 4).

Discussion

Labelling of calcium channels with different 1,4-dihydropyridines in human heart

Our affinity estimates for the binding of 1,4 dihydropyridines to human heart agree with those of other species ([^{3}H]-nimodipine, Janis *et al.*, 1982; [^{3}H]-(+)-PN 200–110, Rengasamy *et al.*, 1985; [^{125}I]-iodipine, Glossmann *et al.*, 1985). It is concluded that the affinity characteristics of ventricular calcium channels are the same in various species including man, as judged by the use of the three radioactive 1,4-dihydropyridines.

Assuming that ligands bind to a homogeneous population of sites in the calcium channel, we would expect the same B_{max} regardless of radioligand used. However, this was not observed. On average [^{3}H]-(+)-PN 200–110 bound to 1.6 fold more sites than [^{3}H]-nimodipine in 8 HOCM patients tested in parallel. [^{125}I]-iodipine bound to 2.24 fold as many sites as [^{3}H]-nimodopine or 1.37 fold as many sites as [^{3}H]-(+)-PN 200–110. Previously it has been suggested that in

Table 3 Binding constants of 1,4-dihydropyridine derivatives in human heart membranes.

Radioligand	*Competing ligand*	Ki (nmol l^{-1})	*Channel blocker*	*Channel activator*
[^{3}H]-(±)-PN 200–110	(+)-PN 200–110	0.14 ± 0.08	+ (a)	
	(−)-PN 200–110	37	?	
	(−)-202–791	6.3	+ (b)	
	(+)-202–791	425		+ (b)
	(±)-Bay K 8644	27 ± 9		+ (c)
	(±)-CGP 28 392	654		+ (d)
[^{3}H]-nimodipine	(+)-PN 200–110	0.20	+ (a)	
	(−)-PN 200–110	24.0	?	
	(±)-Bay K 8644	40.0		+ (c)
[^{125}I]-iodipine	(+)-PN 200–110	0.21	+ (a)	
	(−)-PN 200–110	25.4	?	

(a) Hoff *et al.*, 1984; (b) Kongsamut *et al.*, 1985; (c) Schramm *et al.*, 1983; (d) Laurent *et al.*, 1985.
Ki values are means from two experiments which varied by less than 10%. *Ki* values with s.e.means, means from 3 experiments.

skeletal muscle transverse-tubule membranes (Ferry *et al.*, 1985b) 1,4-dihydropyridines which activate calcium channels bind to lower densities of sites than those which block channels. The rank order for B_{max} values is [^{3}H]-(+)-PN 200–110 > [^{3}H]-nimodipine > [^{125}I]-iodipine ± [^{3}H]-(+)-Bay K 8644.

In heart, however, [^{125}I]-iodipine labelled more sites than [^{3}H]-(+)-PN 200–110, [^{3}H]-nimodipine or [^{3}H]-(±)-Bay K 8644 (guinea-pig, Ferry *et al.*, 1984; human, present paper). Thus, [^{125}I]-iodipine changes position in the rank order in heart compared to skeletal muscle. It is plausible that iodipine is a channel blocker in heart and a partial channel activator in skeletal muscle. This interpretation implies subtle differences between the properties of the calcium channels of heart and skeletal muscle. The rank order of B_{max} values for ligands under the conditions employed (i.e. 37°C) suggests a spectrum between activators and blockers of the calcium channels.

[^{3}H]-(±) Bay K 8644 and unlabelled calcium channel activators as inhibitors of [^{3}H]-(+) 200–110 binding

Recent evidence from patch clamp studies supports the idea that 1,4 dihydropyridines can exert both activating and blocking effects in the calcium channels (Hess *et al.*, 1984). Accordingly, the channel blocker nifedipine can act as a channel activator under appropriate conditions (Schramm *et al.*, 1985). We found no high affinity binding with the activator [^{3}H]-(±)-Bay K 8644 at 37°C in human heart up to 5.5 nmol l^{-1} although high affinity binding in rabbit heart at 15°C with a K_D of 2.5 nmol l^{-1} has previously been reported (Janis *et al.*, 1984). However (±)-Bay K 8644 inhibited [^{3}H]-(+)-PN 200–110 binding with a K_i value of 27 nmol l^{-1} which is close to the K_D of 40 nmol l^{-1} for [^{3}H]-(±)-Bay K 8644 for binding to beating cardiocytes (Bellemann, 1984) at 37°C. Thus, (±)-Bay K 8644 with a K_i value of 27 nmol l^{-1} is a

Table 4 Saturation analysis in four HOCM patients with [^{125}I]-iodipine, [^{3}H]-(+)-PN 200–110, [^{3}H]-nimodipine and [^{3}H]-(−)-dihydroalprenolol

Patient	*β-Adrenoceptors*		*Calcium channel linked 1,4-DHP receptors*					
	[^{3}H]-(−)-dihydroalprenolol		[^{125}I]-iodipine		[^{3}H]-(+)-PN 200–110		[^{3}H]-nimodipine	
	B_{max} (fmol mg^{-1})	K_D (nmol l^{-1})	B_{max} (fmol mg^{-1})	K_D (nmol l^{-1})	B_{max} (fmol mg^{-1})	K_D (nmol l^{-1})	B_{max} (fmol mg^{-1})	K_D (nmol l^{-1})
8/7/84	53	0.93	189	0.28	149	0.14	82	0.10
13/7/84	52	0.73	167	0.26	107	0.06	60	0.08
5/6/84	24	0.68	111	0.23	88	0.06	64	0.15
13/6/84	47	0.67	177	0.21	127	0.06	77	0.13

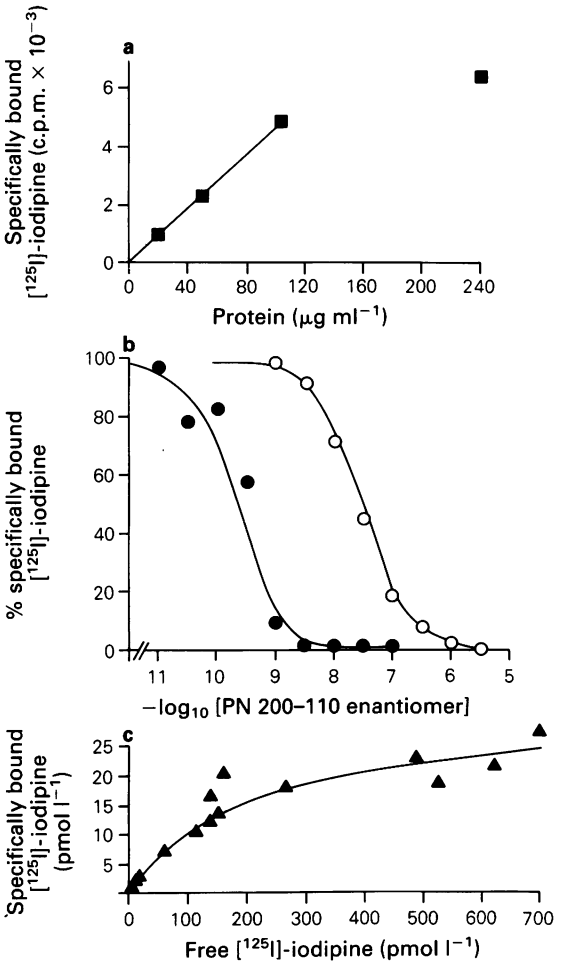

Figure 3 Binding characteristics of [^{125}I]-iodipine to human heart ventricular membranes. (a) Protein dependency: membranes as indicated were incubated with 52 pmol l^{-1} [^{125}I]-iodipine in a volume of 0.25 ml before bound and free radioactivity were separated by rapid filtration. Blanks were defined by 1 μmol l^{-1} (±)-PN 200–110 and have been subtracted. (b) Inhibition of specific [^{125}I]-iodipine binding by (+)-PN 200–110 (●) and (−)-PN 200–110 (○). The concentration of [^{125}I]-iodipine was 52 pmol l^{-1} and protein 31 μg per 0.25 ml. Total bound [^{125}I]-iodipine was 19.7 pmol l^{-1} and blank defined by 1 μmol l^{-1} (±)-PN 200–110 3.7 pmol l^{-1}. Binding constants are given in Table 3. (c) Saturation analysis: conducted in a volume of 0.15 ml with 45 μg protein per assay tube (patient 5/6/84 of Table 4) and a free [^{125}I]-iodipine concentration range of 6–677 pmol l^{-1}. Non-linear fitting revealed a K_D of 233 pmol l^{-1} and B_{max} of 33.3 pmol l^{-1} which corresponds to 111 fmol mg^{-1} of membrane protein. The blank slope was 0.083 pmol l^{-1} (pmol l^{-1})$^{-1}$ free.

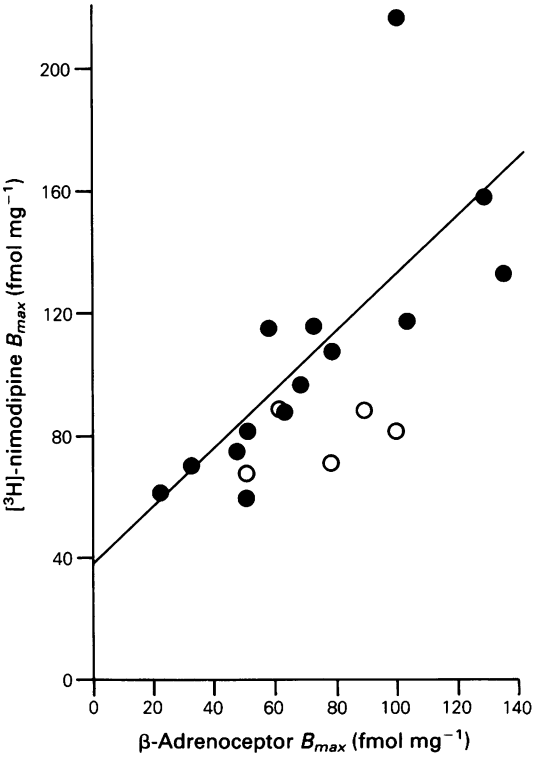

Figure 4 Correlation between the density of β-adrenoceptors and [^{3}H]-nimodipine labelled sites in membranes prepared from 14 HOCM patients (●). The line is fitted by linear regression analysis with a correlation coefficient of 0.73, a y-axis intercept of 39 fmol mg^{-1} and a slope of 0.94. Points from five mitral valve patients are also shown (○).

more potent inhibitor of [^{3}H]-(+)-PN 200–110 binding than the channel activator (+)-(S)-202 791 (Hof *et al.*, 1985; Kongsamut *et al.*, 1985). As racemic 202–791 is a channel blocker (Hof *et al.*, 1985; Kongsamut *et al.*, 1985) and the more potent enantiomer in binding is the channel blocking enantiomer it may be expected that as (±)-Bay K 8644 is a channel activator (Schramm *et al.*, 1983) the enantiomer of higher affinity is a channel activator. This has recently been shown to be the case. (−)-Bay K 8644 is a channel activator, (+)-Bay K 8644 is a channel blocking agent (Franckowiak *et al.*, 1985) and (−)-Bay K 8644 is more potent is binding studies (Bellemann, 1985). The same argument may apply to the channel activator (±)-CGP 28 392 (Laurent *et al.*, 1985).

Marginal presenceof α-adrenoceptors in human ventricular myocardium

When a considerable proportion of myocardial β-adrenoceptors is blocked it is possible to unmask an α-adrenoceptor-mediated positive inotropic effect of physiological catecholamines in a variety of tissues and species (Kaumann, 1970; Wagner & Brodde, 1978; Skomedal *et al.*, 1985; Blinks & Endoh, 1986). However, in isolated preparations of human ventricle, Bruckner *et al.* (1984) observed only an inconstant positive inotropic effect of phenylephrine mediated through α_1-adrenoceptors. Our binding data, showing only a marginal population of α_1-adrenoceptors in human ventricle, are inconsistent with an important role of these receptors in the increase of contractile force with α_1-adrenoceptor agonists. Nawrath & Rupp (1986) reported recently for isolated tissues of human ventricle that the maximum increase in contractile force by phenylephrine is only 15% of the maximum effect caused by β-adrenoceptor stimulation. This observation is in line with our evidence for a small α_1-adrenoceptor population in human ventricle. No α_2-adrenoceptors were labelled with [^{3}H]-rauwolscine, thus, it is unlikely that α_2-adrenoceptors mediate physiological effects of catecholamines in human ventricular myocardium.

Laks & Moraday (1976) have suggested noradrenaline may be a hypertrophy-inducing hormone. In rat heart it has been shown that α_1-adrenoceptors mediate cellular hypertrophy (Simpson, 1985). However, in HOCM, a disease characterized by hypertrophied ventricular cells, only a borderline presence of α_1-adrenoceptors was detected, probably ruling out a contribution to malfunction.

β-Adrenoceptors in human heart

β-Adrenoceptors have previously been labelled in human myocardium with [^{3}H]-(−)-bupranolol (Kaumann *et al.*, 1982), [^{3}H]-(−)-dihydroalprenolol (Bristow *et al.*, 1982), [^{125}I]-(±) hydroxyiodopindolol (Robberecht *et al.*, 1983) and [^{125}I]-(±) iodocyanopindolol (Stiles *et al.*, 1983). Most of the experiments in this study were performed with [^{3}H]-(−)-dihydroalprenolol because this ligand is optically pure, available at high specific activity (102 Ci mmol^{-1}) and extraordinarily long incubation times are not required to reach equilibrium (e.g. [^{125}I]-iodocyanopindolol). The K_D value of 1.4 ± 0.8 nmol l^{-1} in HOCM patients and 1.8 ± 0.6 nmol l^{-1} in mitral valve patients shows that the affinity does not differ in these diseases. The K_D value of [^{125}I]-(−)-iodocyanopindolol were 5 and 6 pmol l^{-1} in two HOCM patients and 6 and 9 pmol l^{-1} in two mitral valve patients again suggesting that affinity of the β-adrenoceptor appears not related to the diseases. Our affinity estimates of [^{3}H]-(−)-dihydroalprenolol and [^{125}I]-(−)-iodocyanopindolol for human heart β-adrenoceptors are higher than those reported by others. Bristow *et al.* (1982) found a 3 to 5 fold lower affinity for [^{3}H]-(−)-dihydroalprenolol, but the heart material, membrane preparation and binding temperature (30°C instead of 37°C) were different. The relatively low affinity of [^{125}I]-(±)-iodocyanopindolol reported by Stiles *et al.* (1983) (K_D = 40 pmol l^{-1}) may be due to the use of the racemate instead of the (−)-enantiomer (see Hoyer *et al.*, 1982), and to incubation times too short to approach equilibrium with the β-adrenoceptors.

Correlation between β-adrenoceptor and calcium channel density HOCM membranes

In the 14 patients with HOCM for which β-adrenoceptor density and calcium channel density was measured a significant correlation was found ($P < 0.01$). The slope of the correlation between β-adrenoceptors and channels is 0.94, which suggests but does not prove a mutual influence of one channel with one receptor. On the other hand, no correlation was seen in the five patients with mitral lesion. Ventricular cells of HOCM patients can have a width up to 100 μm (normal 10–15 μm) (Olsen, 1980; Ferrans, 1982). Cells of HOCM ventricle are thought to exhibit hypercontractility (Goodwin, 1980; Perloff, 1981). The enhanced contractile strength generated by the ventricular cells from HOCM could be due to an enhanced calcium influx and to an increased release of sarcoplasmatic calcium. Because the cell surface area is increased with the square of the radius, considerably more calcium channels per cell would be expected to provide the calcium required for hypercontractility and even for normal contractions. On the other hand, the size of ventricular cells of papillary muscle from mitral lesions is normal or the cells may even be atrophied (Hort & Frenzel, personal communication), which may explain the relatively small amount of putative calcium channels found in these patients.

We would like to suggest that the correlation between β-adrenoceptor density and calcium channel density in HOCM ventricle is due to common pathways of regulation. A recent paper, which appeared after this work had been submitted, is consistent with a more general coregulation of β-adrenoceptors and calcium channels. After chemical denervation of sympathetic nerves with 6-hydroxydopamine, Skattebol & Triggle (1986) found a one third increase in the density of both calcium channels and β-adrenoceptors in rat ventricle.

β-Adrenoceptor activation increases contractile force by increasing intracellular cyclic AMP which in turn activates a cyclic AMP-dependent protein kinase. Injection of the catalytic sub-unit of cyclic AMP-dependent protein kinase into guinea-pig myocytes

increases the inward current through the calcium channel (Osterrieder *et al.*, 1982, Kameyama *et al.*, 1985). In addition, it has been shown that treatment of primary rat heart culture with 8-bromocyclic AMP makes more calcium channels available to open when cells are depolarized (Cachelin *et al.*, 1983). Recently Curtis & Catterall (1985) have shown that the purified calcium channel is phosphorylated *in vitro* by cyclic AMP-dependent protein kinase. Phosphorylation of β-adrenoceptors is also a key step in homologous down-regulation of the receptors (Benovic *et al.*, 1985). The phosporylation and dephosphorylation of both β-adrenoceptors and calcium channels may be crucial processes undergoing common regulation by the sympathetic nervous system.

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