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β-Adrenoceptors of the human myocardium: determination of β_1 and β_2 subtypes by radioligand binding

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1 β-Adrenoceptors of the human myocardium were investigated with binding studies using ^{125}iodocyanopindolol (ICYP) as ligand.

2 Inhibition of ICYP-binding by betaxolol (a selective β_1-antagonist) and ICI 118551 (a selective β_2-blocking drug) resulted in non-linear Scatchard-plots suggesting that both β-adrenoceptor subtypes are present in human left atrium and left ventricle.

3 Computer analysis of the data gave a β_1/β_2-adrenoceptor ratio of approximately 65 : 35 both for left atrium and for left ventricle.

Introduction

Over the past few years, evidence has been accumulating that β_1 and β_2-adrenoceptors are not only organ-specifically distributed, but that both β-adrenoceptor subtypes can coexist in a single organ, especially the heart, of various species.

In addition to pharmacological studies (Ablad, Carlsson & Ek, 1973; Carlsson, Dahlof, Hedberg, Persson & Tangstrad, 1977) radioligand binding studies revealed this coexistence. Hedberg, Minneman & Molinoff (1980) showed that β_1 and β_2-adrenoceptors were present in cat and guinea-pig right atria while the left ventricle of both species contained only β_1-adrenoceptors. Engel, Hoyer, Berthold & Wagner (1981) confirmed this result for guinea-pig left ventricle. Similarly, Brodde, Leifert & Krehl (1982) reported that β_1 and β_2-subtypes coexist in rabbit atria while the adrenoceptors of the ventricles are predominantly β_1.

On the other hand, relatively little is known about the adrenoceptors of human myocardium. Most experiments to date have been performed on the right atria (Harms, 1976; Schümann, Wagner, Knorr, Reidemeister & Sadony, 1978; Wagner, Schümann, Knorr, Rohm & Reidemeister, 1980), tissue which is routinely excised during open heart surgery. In contrast, human ventricular myocardium is only rarely available. The aim of the present study was to determine the quantities of β_1 and β_2-adrenoceptors in human left atrium and left ventricle. For this purpose, we used the same method as for the determination of β-adrenoceptors in pig coronary arteries (Schwartz & Velly, 1983). We examined the inhibition of ^{125}iodocyanopindolol (ICYP) binding by the β_1-selective drug, betaxolol, and the β_2-selective drug, ICI 118551 (erythro-DL-1(7-methylindan-4-yloxy)3 isopropyl-aminobutan-2-ol).

ICYP proved a good ligand for studying β-adrenoceptors. Compared to tritiated compounds ([^{3}H]-dihydroalprenolol, [^{3}H]-DHA), it has a very high specific activity (about 2000 Ci mmol^{-1} versus 20 to 60 Ci mmol^{-1} for [^{3}H]-DHA). ICYP is more selective than iodohydroxybenzylpindolol (IHYP) reported to have an important affinity for α-adrenoceptors (Sporn & Molinoff, 1976) and 5-hydroxytryptamine (5-HT) receptors (Dickinson, Nahorski & Willcocks, 1981). ICYP binds to β_1 and β_2-adrenoceptors with equal affinity (Engel *et al.*, 1981). Analysis of the displacement curves of this ligand by selective β_1 or β_2 agents reveals the presence of one or both types of β-receptors and the relative proportions of each receptor subtype. The coexistence in a tissue of both β_1 and β_2-receptors results in curvilinear modified Scatchard plots.

Methods

Membrane preparation

Four hearts obtained soon after death were frozen for at least 24 h. Membranes from left atria and left ventricles were prepared according to the technique

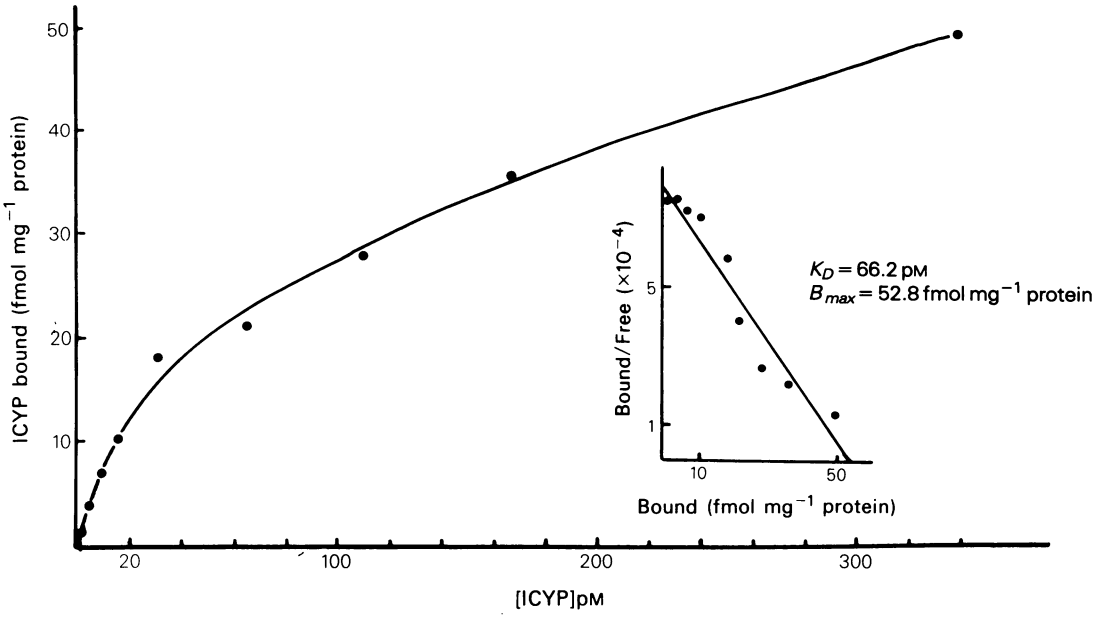

Figure 1 A typical single experiment of specific ^{125}iodocyanopindolol (ICYP) binding to human left atrium. The inset shows a Scatchard-plot. Mean K_D and B_{max} values were respectively 66 ± 7 pM and 43 ± 5 fmol mg^{-1} protein (mean of 6 experiments done on cardiac tissue from four different hearts).

of Minneman, Hegstrand & Molinoff (1979). Frozen tissues were thawed and dissected on ice; they were homogenized with a Polytron (setting 6 for 30 s) in 20 vol. (w/vol) of 20 mM Tris-HCl, pH 7.5 buffer containing 0.9% NaCl (isosaline buffer). The homogenates were centrifuged at 20,000 *g* for 10 min and the supernatants discarded; the pellets were resuspended in isosaline buffer to give a protein

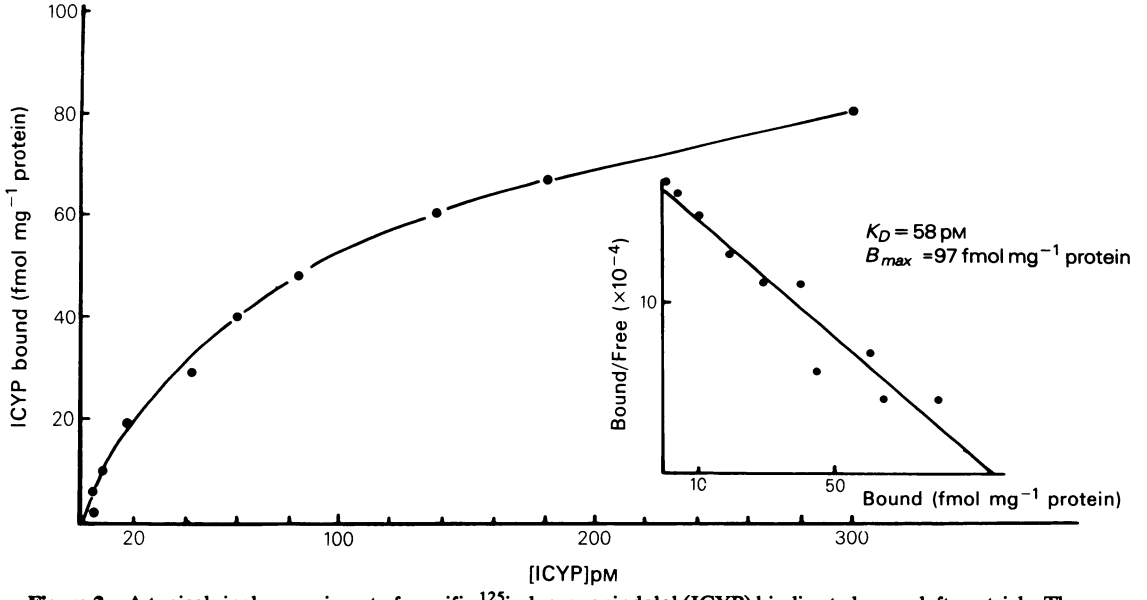

Figure 2 A typical single experiment of specific ^{125}iodocyanopindolol (ICYP) binding to human left ventricle. The inset shows a Scatchard-plot. Mean K_D and B_{max} values were respectively 56 ± 5 pM and 76 ± 8 fmol mg^{-1} protein (mean of 8 experiments done on cardiac tissue from four different hearts).

content of 333 μg ml^{-1}. Approximately 100 mg of protein were obtained from 1 g of human heart atrium or ventricle. Protein concentration was determined by the method of Lowry, Rosebrough, Far & Randall (1951).

Membranes from guinea-pig left ventricle and from rat cerebellum were prepared according to the method of Minneman *et al.* (1979).

Preparation of ICYP

^{125}Iodocyanopindolol was synthesized according to the method described by Engel *et al.*, (1981).

Binding assay

Binding assay was carried out as described by Engel *et al.* (1981): 150 μl of the membrane suspension containing 50 μg protein, 50 μl of ICYP (30–40000 ct min^{-1}) and 50 μl of the competing drug at various concentrations were incubated for 55 min at 37°C in 10 mM Tris-HCl, pH 7.4 containing 0.154 M NaCl and 1.1 mM ascorbic acid. Bound and free ligand were separated by rapid filtration through Whatman GF/B filters. Each filter was rapidly washed with an additional volume of 15 ml of the same buffer solution. The radioactivity of the filters was measured in an Autogamma Packard counter at 70% counting efficiency. Specific binding of the ligand was defined as the amount of the label bound in the absence of competing ligand minus the amount bound in the presence of (±)-propranolol 10^{-5} M. Specific binding was never less than 90% of total binding. For determination of the dissociation constant (K_D) and of the maximal number of binding sites (B_{max}), saturation experiments were performed by incubating 150 μl of membrane preparation with 50 μl of increasing concentrations (4 to 250 pM) of ICYP with and without (±)-propranolol 10^{-5} M. Assay conditions were as described above.

Analysis of data

The experimental data given in the paper are means ± s.e.mean. The equilibrium dissociation constant (K_D) and the maximal number of binding sites (B_{max}) were calculated from plots according to Scatchard. For the identification of the myocardial receptors, the concentration-inhibition curves were transformed into modified Scatchard plots, i.e. by plotting % inhibition of binding versus % inhibition divided by the concentration of the competing agent. By analysing competition curves with a computer modelling technique (SAAM program; Berman & Weiss, 1967; Berman, 1968) the proportions of β_1 and β_2-adrenoceptors and the K_D of betaxolol and ICI 118551 for each receptor subtype can be deter-

Table 1 Comparison of dissociation constants of betaxolol and ICI 118551 obtained from human left atrium and ventricle, from guinea-pig left ventricle and from mature rat cerebellum

Compound	*Human left atrium*				*Human left ventricle*				*Guinea-pig left ventricle*	*Mature rat cerebellum*	
	Subtypes % β_1/β_2	K_D (M) β_1	β_2	*Selectivity* β_1/β_2	*Subtypes %* β_1/β_2	K_D (M) β_1	β_2	*Selectivity* β_1/β_2	K_D (M)*	K_D (M)*	*Selectivity* β_1/β_2
Betaxolol	62/38	$4.4.10^{-8}$ ±0.07	$8.9.10^{-6}$ ±4	202	64/36	$1.9.10^{-8}$ ±0.4	$7.8.10^{-6}$ ±3.6	410	$0.6.10^{-8}$ ±0.04	$1.2.10^{-6}$ ±0.2	200
ICI 118551	64/36	$3.8.10^{-6}$ ±0.9	$6.5.10^{-8}$ ±1.4	0.017	65/35	$3.5.10^{-6}$ ±0.5	$6.9.10^{-8}$ ±3.4	0.02	8.10^{-8} ±0.9	$0.9.10^{-8}$ ±0.2	0.11

*Unpublished results; values are means ± s.e.mean of five experiments done in triplicate. Membranes were prepared according to Minneman, Hegstrand & Molinoff (1979). Binding assays were performed by the method described for human left atrium and ventricle.

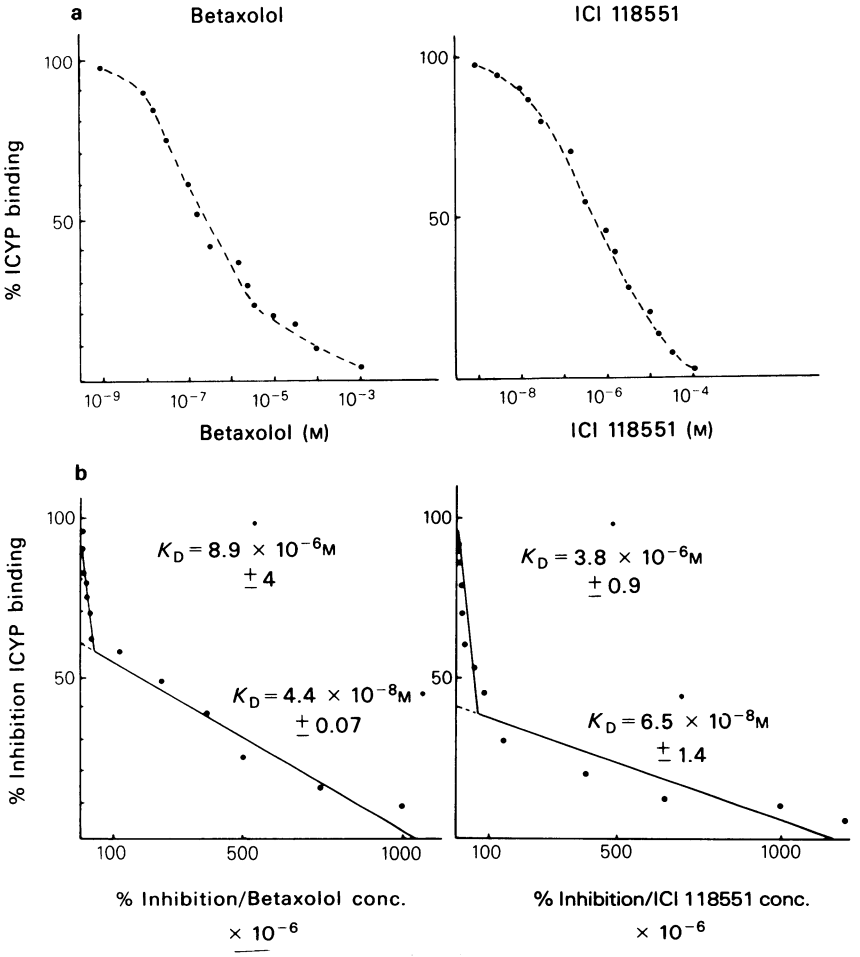

Figure 3 (a) Inhibition curves of ^{125}iodocyanopindolol (ICYP) binding to human left atrium by betaxolol and ICI 118551. Each point is the mean of five experiments done in triplicate with preparations from four different hearts. (b) Modified Scatchard-plot. K_D values are means ± s.e.mean of five experiments and are obtained by analysing competition curves with the computer modelling technique.

mined. This technique enables an accurate evaluation of the proportions of the two receptor subtypes but involves one major assumption, namely that there are only two β-adrenoceptor subtypes.

Results

ICYP binding to human left atrium and left ventricle

Specific ICYP binding to membranes from human left atrium and left ventricle increased linearly with increasing membrane concentrations ranging from 10 to 100 μg protein per assay.

Binding assays were all performed at a concentration of 50 μg protein per assay. Specific ICYP binding rose with increasing ICYP concentrations ranging from 2 to 300 pM. Typical binding experiments are shown in Figures 1 and 2. Scatchard analysis of these data gave linear plots suggesting a single class of binding sites. For left atria, mean K_D value, calculated by linear regression analysis was 66 ± 7 pM and the maximal number of binding sites (B_{max}) 43 ± 5 fmol mg^{-1} protein (mean of 6 experiments done on cardiac tissues from four different hearts). For left ventricle, mean K_D value was 56 ± 5 pM and B_{max} 76 ± 8 fmol mg^{-1} protein (mean of 8 experiments done on cardiac tissues from four different hearts).

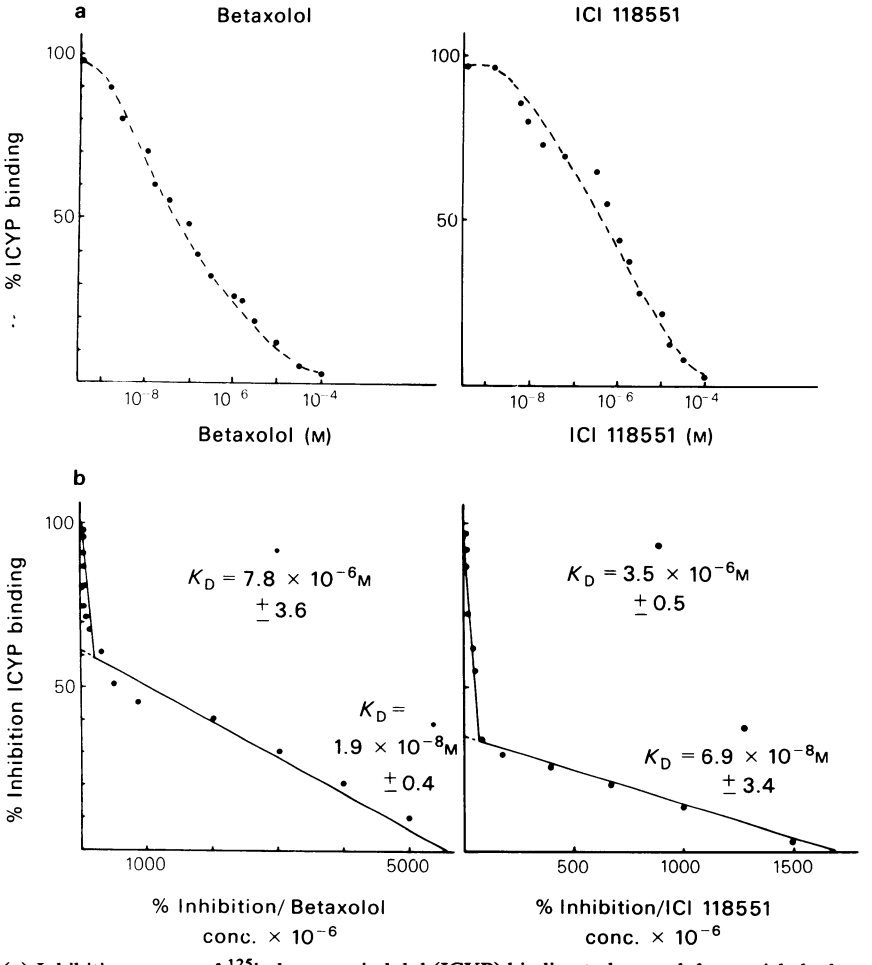

Figure 4 (a) Inhibition curves of ^{125}iodocyanopindolol (ICYP) binding to human left ventricle by betaxolol and ICI 118551. Each point is the mean of five experiments done in triplicate with preparations from four different hearts. (b) Modified Scatchard-plot. K_D values are means ± s.e.mean of five experiments and are obtained by analysing competition curves with the computer modelling technique.

Inhibition of ICYP binding by drugs acting selectively on β_1 and β_2-adrenoceptors

Figures 3 and 4 show, for left atrium and left ventricle, the inhibition curves of ICYP binding by betaxolol (β_1 selective) and ICI 118551 (β_2-selective) and the transformation of these curves into modified Scatchard plots. Scatchard plots can be best described by two distinct straight lines for each agent, indicating the presence of two binding sites, one of low affinity and the other of hi ed finity. For betaxolol, the high affinity component is related to β_1-adrenoceptors. Inversely, for ICI 118551, the high affinity component is related to β_2-adrenoceptors. Computer modelling of these curves according to a two class model, gives the percentage of β_1 and β_2-adrenoceptors and dissociation constants of the two drugs for each receptor subtype. The percentage distribution of β_1 versus β_2-adrenoceptors for atrium was 62:38 with betaxolol, and 64:36 with ICI 118551. For ventricle, the percentages were 64:36 with betaxolol, and 65:35 with ICI 118551.

Table 1 gives results obtained on human left atrium and left ventricle with betaxolol and ICI 118551 compared with those we obtained with the same agents on guinea-pig left ventricle, which is considered to contain only β_1-adrenoceptors and on mature rat cerebellum, which is considered to contain only β_2-adrenoceptors (unpublished results).

Discussion

Analysis of these data shows relatively good correlation between the selectivities calculated from affinities observed with tissue containing only one β-receptor subtype and those observed with the tissues where β_1 and β_2-adrenoceptors coexist. It should be noted that the β_2-selectivity we observed for ICI 118551 in binding assays on guinea-pig left ventricle and mature rat cerebellum was only 9; O'Donnell & Wanstall (1980) evaluating β_2-selectivity from pA_2 values obtained on guinea-pig trachea and atria, found a value of 54 whilst Bilski, Dorries, Fitzgerald, Jessup, Tucker & Wale (1979) assessing β_2-selectivity from pA_2 values obtained on guinea-pig uterus and atria, found a selectivity of 123. The β_2-selectivity we observed for ICI 118551 with human left atrium and left ventricle tally better with the result of O'Donnell & Wanstall (1980).

Furthermore, our results enable us to conclude that β_1- and β_2-adrenoceptors coexist in the left atrium and left ventricle of human heart. ICYP is a good ligand for this kind of study: it binds to β_1- and β_2-adrenoceptors with equally high affinity (Engel *et al.*, 1981) and has very low non-specific binding. With betaxolol, the proportion of high affinity sites (β_1) is 62% for left atrium and 64% for left ventricle; ICI 118551 produces an exact mirror image, the proportions of high affinity sites (β_2) being respectively 36% for atrium and 35% for ventricle. The use of two different drugs with different affinities and opposite selectivities yielded the same percentage of β_1 and β_2-adrenoceptors, which is consistent with the assumption that there are two receptor subtypes here.

Other authors have found proportions of β_1- and β_2-adrenoceptors in the human heart which partially tally with our results. In an abstract, Stiles, Taylor & Lefkowitz (1982) report that β_1-adrenoceptors predominate in human left ventricular myocardium ($> 65\%$). They found that tissue obtained more than 6 h after death contained mostly β_2-adrenoceptors, which suggests that β_1-receptors are more labile. This could perhaps explain discrepancies observed in the results obtained by various workers. Brodde, Karad, Zerkowski, Rohm & Reidelmeister (1982), found that about 20% of β-adrenoceptors in human right atrium are of the β_2-subtype and in right ventricle, about 10%. In fact, we cannot exclude the possibility of inter-individual variations. As regards the relative distribution of β_1- and β_2-adrenoceptors, our results obtained with normal hearts, showed no significant variations, but the possibility must be considered that genetic, environmental and physiopathological factors may control the ratio of β_1- and β_2-receptors in the human heart. As early as 1975, Vaughan Williams, Raine, Cabrera & Whyte (1975) showed that in rabbits, although practolol blocked β-receptors longer than propranolol, in some animals, the blockade was incomplete after practolol, which suggests that β_2-receptors coexisted with β_1 only in 40% of the animals.

However, our technique involves one important restriction: myocardial preparations necessarily include vascular receptors, especially those from penetrating transmural vessels. On the other hand, in human left ventricle we found a binding site concentration twice that found for left atrium. Baker & Potter (1980) described the same disparity in the heart of dogs and rats and noticed that this distribution was very closely parallel to that of blood flow. On the other hand, β-adrenoceptors are not distributed like the adrenergic innervation; noradrenaline content is higher in atrium than in ventricle, as has been reported by Chidsey & Braunwald (1966) who, in the human heart, found 1.77 $\mu g\,g^{-1}$ noradrenaline in the atrium versus only 0.36 $\mu g\,g^{-1}$ in the left ventricle. Baker *et al.* (1980) interpreted their data as evidence that most cardiac adrenoceptors are not located at synapses. Furthermore, we found identical proportions of β_1 and β_2-adrenoceptors in left atrium and left ventricle which have not the same adrenergic innervation; this does not fit in with the hypotheses of Bryan, Cole, O'Donnell & Wanstall (1981) that β_1-adrenoceptors might be 'innervated' receptors associated with noradrenergic nerves and that β_2-adrenoceptors might be 'non-innervated' receptors associated with extraneuronal uptake.

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