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FACTORS CONTROLLING β_1-ADRENOCEPTOR AFFINITY AND SELECTIVITY

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Abstract—A membrane preparation of the calf heart left ventricle has been used after identification and characterization, as a source of myocardial β_1-adrenoceptors for radioligand binding studies. The displacement of specifically bound (−)-[^{3}H]dihydroalprenolol by some β-adrenoceptor ligands appeared to be pH-dependent, which could be related to the ionization characteristics of the compounds. Among the usually four ionic species of the ligand, present at physiological pH, the cation was shown to govern β_1-adrenoceptor affinity. Furthermore, quantitative structure affinity relationships for the interaction with β_1- and β_2-adrenoceptors were established for the phenoxypropanolamines, a class of β-adrenoceptor ligands. The *N*-isopropyl-oxypropanolamine side chain itself does not discriminate between β_1- and β_2-adrenoceptors, whereas aromatic substitution *ortho* to the side chain induces some β_2-selectivity. Selectivity for myocardial β_1-adrenoceptors is mainly obtained by aromatic substitution *para* to the side chain. This substitution pattern yields a decrease in β_2-adrenoceptor affinity, far more pronounced than the decrease in β_1-adrenoceptor affinity.

The myocardial β-adrenoceptors are of prime interest for our understanding of cardiovascular functions and diseases. These receptors are the 'sites of action' for major classes of drugs, i.e. β-adrenoceptor agonists and antagonists, useful as positive inotropic, and both antianginal and antihypertensive agents, respectively. The introduction of the radioligand binding technique has largely contributed to current knowledge of the interaction between the β-adrenoceptor and its ligands [1].

The assessment, however, of structural features of β-adrenoceptor ligands required for a selective interaction with myocardial β-adrenoceptors, has been based mainly on results obtained from *in vivo* experiments or functional studies on isolated heart tissues [2, 3]. Apart from usually obscuring factors in these types of studies like receptor reserve, pharmacokinetic behaviour and metabolic aspects, the compensatory properties of the cardiovascular system in order to maintain homeostasis, yield further difficulties in the evaluation of the ligand–receptor interaction. To largely overcome these problems, we have used a membrane preparation of the calf heart left ventricle, and determined the binding of (−)[^{3}H]-dihydroalprenolol and its displacement by various ligands in order to characterize the preparation used and to derive clues, essential for myocardial β-adrenoceptor affinity and selectivity.

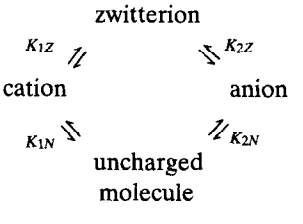
MATERIALS AND METHODS

Ionization schemes

Most β-adrenoceptor agonists are dibasic acids with 'overlapping' macroscopic ionization constants K_1 and K_2, yielding the following ionization schemes:

$$\text{cation} \overset{K_1}{\rightleftharpoons} \text{zwitterion} \overset{K_2}{\rightleftharpoons} \text{anion}$$

uncharged molecule



Recently, the determination of K_{1Z}, K_{2Z}, K_{1N} and K_{2N}, the four microscopic ionization constants, was described [4]. Briefly, K_1 and K_2 are found by electrochemical titration, K_{1Z} is estimated by u.v.-spectrophotometry and the three other microscopic ionization constants are then calculated. The knowledge of these four microscopic ionization constants permits the calculation of the relative distribution of

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Abbreviations: HEPES, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid; [^{3}H]DHA, (−)-[^{3}H]-dihydroalprenolol; cAMP, cyclic 3′,5′-adenosine monophosphoric acid; EPPS, *N*-[2-hydroxyethyl]-1-piperazine-propanesulfonic acid; MIX, methylisobutylxanthine; ATP, adenosine-triphosphate; TRIS, tris(hydroxymethyl) aminomethane; EDTA, ethylenediaminetetraacetic acid; GppNHp, guanylyl-5′-imidodiphosphate; m., musculus; fmol, 10^{-15} mole.

the four ionic species (cations, zwitterions, uncharged molecules and anions) at any given pH [5]. The ionization of most β-adrenoceptor antagonists, usually monobasic acids, is less complicated, resulting in an equilibrium between cation and uncharged molecule only. Measurements have been performed at 25°. For some derivatives ionization constants have been determined at 37° as well. Although K_2 proved to vary considerably, it also turned out that K_1 (and thus f_c, see text, Table 3 and Fig. 2) remained virtually identical at both temperatures.

Preparation of membranes

Calf hearts were cleared from fat, immediately after killing of the animals at the Amsterdam abattoir. The left ventricles were separated, cut into pieces and transported on ice in buffer (5mM HEPES, 140 mM NaCl, pH = 7.5 at 20°) to the laboratories within 30 min. Homogenization, differential centrifugation and storage were fully according to a previously described procedure, developed for a bovine skeletal muscle preparation [6]. Binding and adenylate cyclase characteristics remained constant for more than half a year.

[^{3}H]DHA binding assay

All assays were performed as previously described [6], except for the [^{3}H]DHA concentrations used: now 0–18 nM in saturation experiments, and 0.5–1.0 nM in displacement studies. Specific binding was defined as the difference between radioactivity bound in the absence and presence of 1.10^{-5} M (−)-isoprenaline.

cAMP assay

All assays were performed in duplicate in a final volume of 350 μl. All following solutes were dissolved in buffer (20 mM EPPS, 10 mM $MgCl_2.6H_2O$ 120 mM NaCl, pH = 8.0 at 20°): (a) 50 μl MIX (final concentration 5×10^{-4} M); (b) 50 μl ATP (final concentration 1×10^{-3} M); (c) 100 μl membrane suspension (2 mg protein/ml); (d) 150 μl solution of various compounds known to stimulate the adenylate cyclase. All reactions were carried out at 37° for 15 min, and terminated by dilution with cold buffer (50 mM TRIS, 4 mM EDTA, pH = 7.5 at 20°) followed by heating at 95° for 3 min. The cAMP content was determined by an assay with a cAMP binding protein (purified from bovine muscle).

Protein determination

Protein was determined by the method of Lowry *et al.* using bovine serum albumin as a standard [7].

Data analysis

The method of computer assisted data analysis following the law of mass-action has been previously described [6]. K_D-values are given with approximated S.E.

QSAR parameters

Log P. Log P values (octanol/water) of the aromatic moiety of the ligands (i.e. the corresponding, substituted benzenes) were calculated, according to the hydrophobic fragmental system [8].

Steric parameters. The steric branching parameter (S_b) for all substituents was calculated according to Austel *et al.* [9].

Multiple regression analysis. Computer assisted multiple regression analyses were performed, which yielded the regression equations together with statistic parameters, adjusted for the degrees of freedom. The regression coefficients are given with their standard errors.

Materials

(−)-[^{3}H]DHA (71–104 Ci/mmole) was purchased from Amersham (Bucks, U.K.) and New England Nuclear (Dreieich, FRG). The cAMP binding protein assay was from Amersham. (−)-Isoprenaline (hydrochloride), (+)-isoprenaline, (−)-noradrenaline, (−)-adrenaline (all hydrogentartrates) and GppNHp (Na-salt) were obtained from Sigma (St. Louis, MO). (−)- and (+)-Propranolol, (±)-pronethalol (all hydrochlorides) and (±)-practolol (base, ICI, Macclesfield, Cheshire), (±)-pirbuterol (dihydrochloride, Pfizer, Groton, CT), (±)-AH3474 (hydrochloride, Allenburys/Glaxo, Ware, Herts.), terbutaline (sulphate, Astra, Södertälje, Sweden), (−)-L643,717[2-{p-(3,4dimethoxyphenethylamino)-2-hydroxypropoxy]-phenyl}-4-(2-thienyl)imidazole] (dihydrochloride, Merck Sharp & Dohme, West Point. PA), (±)-betaxolol (hydrochloride, Synthélabo, Paris), (−)-alprenolol and (±)prenalterol (hydrochlorides, Hässle, Mölndal, Sweden) and all Kö-compounds (hydrochlorides, Boehringer, Ingelheim, FRG) were gifts. The unsubstituted phenoxypropanolamine derivative (±) and (±)-VUF 8303 [*N*-*t*-butyl-2-(3,5-dichloro-4-hydroxyphenyl)-2-hydroxyethylamine] (hydrochlorides) were synthesized in our laboratories [10, 11]. All other chemicals were of analytical grade.

RESULTS AND DISCUSSION

Identification and characterization of calf heart left ventricle β-adrenoceptors

The characteristics of the β-adrenoceptors present on membrane particles of the left ventricle of the calf heart are graphically represented in Fig. 1a–d. Thus, in Fig. 1a [^{3}H]DHA specific binding sites display high affinity and low capacity (K_D = 1.80 ± 0.10 nM, B_{max} = 156.9 ± 4.0 fmoles/mg protein at pH = 7.5, and K_D = 1.40 ± 0.07 nM, B_{max} = 184.6 ± 4.8 fmoles/mg protein at pH = 8.3). A marked stereoselectivity is found for the binding sites (+/− ratio for propranolol = 86, for isoprenaline = 75, see Table 1), which is a prerequisite for β-adrenoceptors (Fig. 1b). The rank order of the three catecholamines (isoprenaline > adrenaline ⩾ noradrenaline) is suggestive for the β_1-nature of these binding sites (Fig. 1c). The displacement of [^{3}H]DHA by L643,717, a highly β_1-selective ligand which has recently been described [12], favours this suggestion: 90% of the binding sites has high affinity for this selective ligand (Fig. 1d, see also Table 1). Stimulation of the enzyme adenylate cyclase in this preparation results in a limited production of cAMP, achieved by the addition of a β-adrenoceptor agonist, GppNHp or NaF (Table 2). Surprisingly, the stimulation by NaF is less effective than by isoprenaline

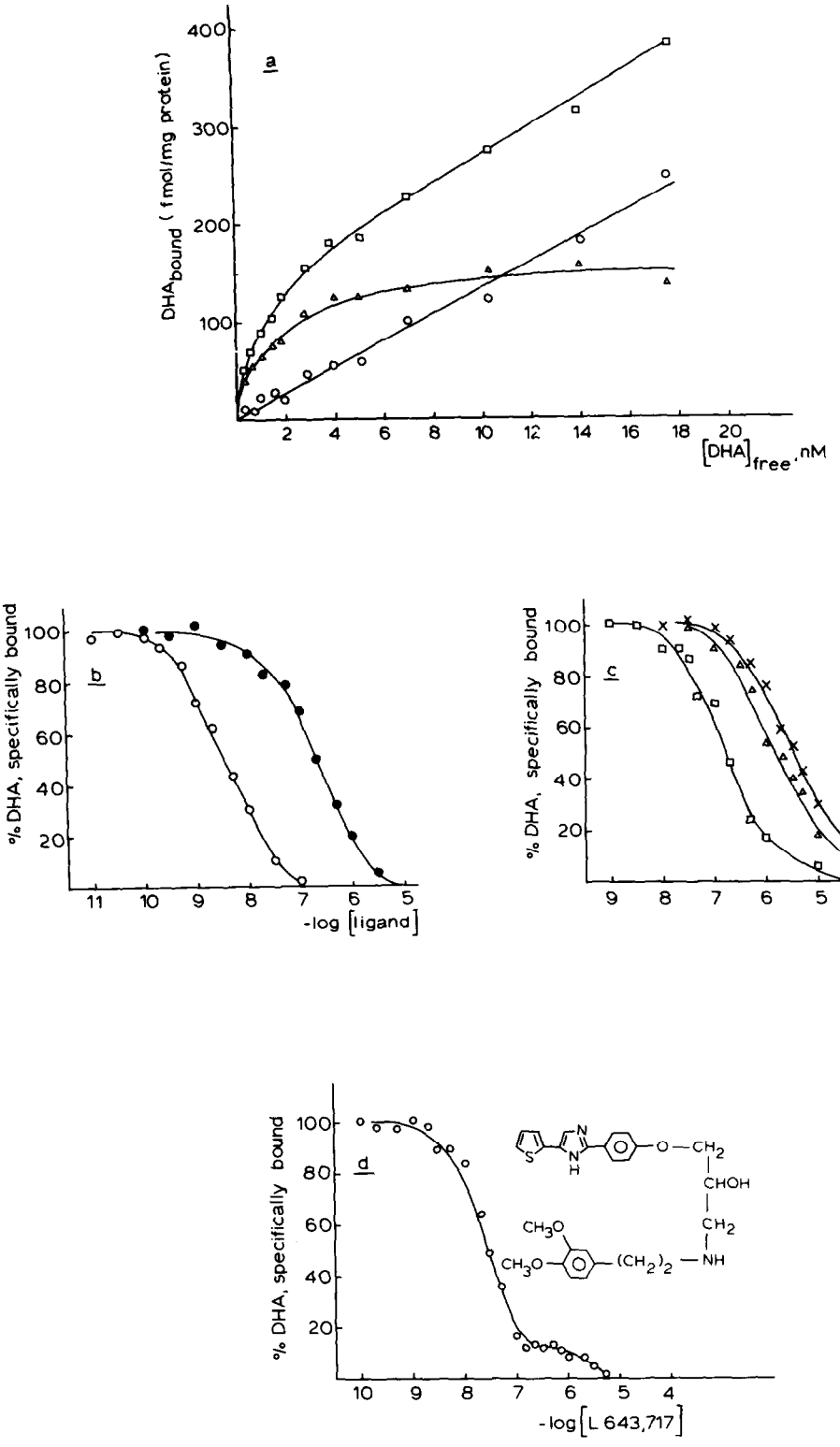


Fig. 1. (a) [^{3}H]DHA binding to membrane particles of the calf heart left ventricle (pH = 7.5, representative experiment): □ total binding, ○ non-specific binding, △ specific binding. (b) Displacement curves (pH = 7.5, representative experiment) of (−)-propranolol (○) and (+)-propranolol (●); [^{3}H]DHA = 0.98 nM. (c) Displacement curves of the three catecholamines (−)-isoprenaline (□), (−)-adrenaline (△), (−)-noradrenaline (×), no GppNHp added (pH = 7.5, representative experiment); [^{3}H]DHA = 0.58 nM. (d) Displacement curve of L643,717, (structural formula shown), a β_1-selective ligand, no GppNHp added (pH = 7.5, representative experiment); [^{3}H]DHA = 0.83 nM.

Table 1. K_D-values (nM, ± approximated S.E., pH = 7.5, N = 2–5) for various β-adrenoceptor ligands as determined by computer-assisted data-analysis. The experiments were performed in the absence of GppNHp

	K_D(nM)
(−)-propranolol	1.2 ± 0.1
(+)-propranolol	103 ± 12
(−)-isoprenaline	54 ± 5
(+)-isoprenaline	4053 ± 450
(−)-adrenaline	1900 ± 190
(−)-noradrenaline	2190 ± 330
(−)-L643,717	2.3 ± 0.7 90% (β_1)
	455 ± 177 10% (β_2)

(with or without GppNHp). The reverse is encountered too, e.g. in the ventricular myocardium of the rat [13]. Furthermore, the low stimulation by isoprenaline and/or GppNHp parallels the negligible influence of GppNHp on the displacement of [^{3}H]-DHA by β-adrenoceptor agonists. The respective displacement curves in the presence (10^{-4} M) and absence of GppNHp proved to be superimposable for all three catecholamines. These findings sharply contrast with similar experiments performed on a skeletal muscle membrane preparation, the bovine m. trapezius, containing β_2-adrenoceptors exclusively [6]. In the latter case, two binding states for agonists with high and low affinity could be discriminated in the absence of GppNHp, whereas in the presence of GppNHp (10^{-4} M) the displacement curves were remarkably steepened, yielding the low affinity state only. With the same membrane preparation an abundant production of cAMP was noticed after stimulation of the enzyme adenylate cyclase. The reason(s) for these discrepancies between the calf heart and bovine m. trapezius membrane preparations is/are not clear. As the preparation procedure is identical for both systems, it seems likely that different functional aspects are involved, e.g. the amounts of N_s, the stimulatory guanine nucleotide-binding regulatory protein in both preparations.

We may safely conclude, however, that the left ventricle membrane preparation of the calf heart is a rich pool of predominantly β_1-adrenoceptors, coupled, although poorly, to the enzyme adenylate cyclase.

Table 2. cAMP production (pmoles/mg protein/min) by stimulation of the adenylate cyclase of the left ventricle of the calf heart. Results are the values of two experiments, both performed in duplicate, incubation time 15 min

	cAMP
Basal	26.9–32.9
NaF (10^{-2} M)	43.0–43.2
GppNHp (10^{-4} M)	52.2–55.4
Isoprenaline (10^{-4} M)	55.4–61.4
Isoprenaline (10^{-4} M) + GppNHp (10^{-4} M)	66.9–67.4

The ionic species interacting with the β_1-adrenoceptor

The interaction between [^{3}H]DHA and the calf heart β-adrenoceptors is slightly favoured at higher pH (cf. K_D = 1.4 nM at pH 8.3 and 1.8 nM at pH 7.5). Analogous to results obtained on the skeletal muscle preparation (β_2) [6], we again noticed a pH-dependent shift of the [^{3}H]DHA displacement curves of several β-adrenoceptor ligands (antagonists and agonists) to lower affinity with increasing pH, in contrast to the trend observed with [^{3}H]DHA. In Table 3, the K_D-values of a number of derivatives are given for the two pH-values studied, together with the structural formulas. Furthermore, in this table, the relative fractions of cations, as a percentage of the 'total drug concentration', at pH = 7.5 and pH = 8.3 are included, derived from the ionization schemes of the compounds. Whereas [^{3}H]-DHA (dihydroalprenolol), propranolol and pronethalol show only minimal variation in the amounts of cations, the four other compounds, all with an ionizable phenolic hydroxygroup, do show a rather extensive fluctuation. The decrease in amounts of cations with increasing pH, especially shown by AH3474, VUF 8303 and pirbuterol closely parallels the decrease in affinity of these ligands. This finding qualitatively suggests that only cations among the four ionic species possible at physiological pH are interacting with the calf heart β-adrenoceptors. Expressing the results, given in Table 3, quantitatively, we reached the same conclusion. The percentage changes in the concentration of cations in the pH-region 7.5–8.3 were plotted against the percentage changes in K_D-values within this pH-range for all seven ligands. The linear dependency between the two parameters, as shown in Fig. 2, demonstrates that one common factor governs the changes in K_D, namely the variation in the concentration of cations.

As a similar relationship was established for the β_2-adrenoceptor previously [6], it now appears that the same holds true for the β_1-adrenoceptor, present in excess on the calf heart membrane preparation.

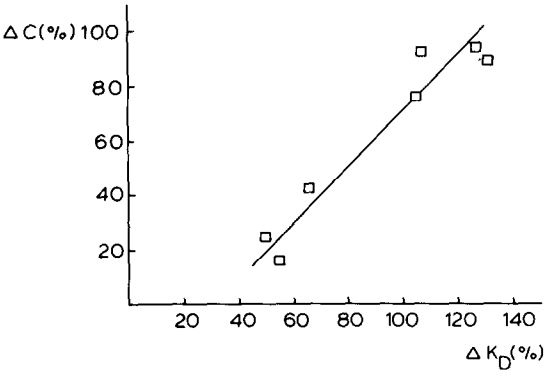
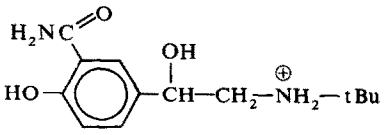
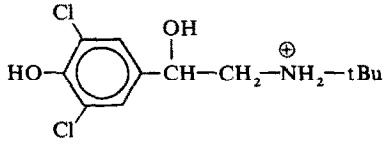
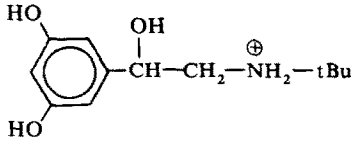
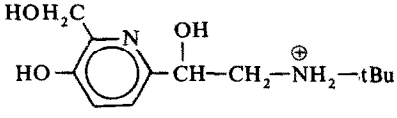
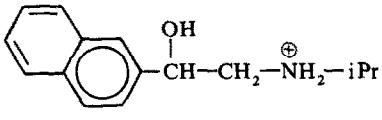
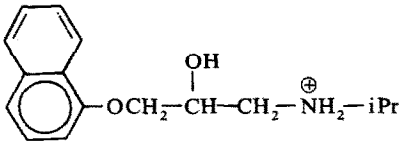
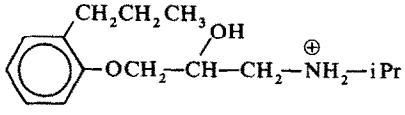


Fig. 2. Relation between ΔK_D and Δc (r = 0.9563), both expressed in %:

$$\Delta c = \frac{\text{fraction of cations present at pH = 8.3}}{\text{fraction of cations present at pH = 7.5}}$$

$$\Delta K_D = \frac{K_D\text{-value at pH = 7.5}}{K_D\text{-value at pH = 8.3}}$$

Table 3. K_D-values* of some β-adrenoceptor ligands (structures given) at different pH-values, together with the relative fractions of cations† (f_c) at these pH-values

	Structures	pH	K_D (μM)	f_c(%)
AH 3474		7.5	3.18 ± 0.27	39.8
		8.3	6.40 ± 0.35	9.4
VUF 8303		7.5	5.33 ± 0.23	3.7
		8.3	9.88 ± 0.41	0.6
Terbutaline		7.5	40.5 ± 3.5	94.1
		8.3	39.1 ± 2.9	71.5
Pirbuterol		7.5	6.48 ± 0.62	73.8
		8.3	9.98 ± 0.59	30.9
Pronethalol		7.5	0.18 ± 0.02	98.5
		8.3	0.17 ± 0.02	91.1
Propranolol		7.5	0.0035 ± 0.0006	98.8
		8.3	0.0028 ± 0.0002	92.8
Dihydroalprenolol		7.5	0.0018 ± 0.0001	98.0
		8.3	0.0014 ± 0.0001	88.6

* Determined in HEPES buffer (HEPES 20 mM, $MgCl_2.6H_2O$ 10 mM, NaCl 120 mM) at 37° ($N = 3$).
† Determined in 0.1 M KCl at 25°, in %.

From studies on isolated organ preparations no such unanimous judgment could be pronounced. Dose–response relationships of adrenaline to positive inotropic activity of the turtle heart proved to be pH-dependent in a way similar as described above, suggesting the adrenaline-cation responsible for activity [14]. On the other hand, more recently, pH-dependent changes in dose–response relationships of noradrenaline were observed on isolated rat atria in a pH-region, where the fraction of cations hardly changes, suggesting the involvement of one of the other ionic species [15]. One of the implications of our results is the 'hidden' high affinity of e.g. VUF 8303. Corrected for the amount of cations present at pH = 7.5 the 'true' K_D-value of the compound is 0.20 μM, considerably lower (representing higher affinity) than the tabulated value of 5.33 μM. Generally, "total cation concentration" rather than "total drug concentration" should be the preferred parameter for biological activity in the β-adrenergic field.

Structural requirements for β-adrenoceptor subtype selectivity

The availability of two membrane preparations with (almost) homogeneous populations of β-adrenoceptor subtypes allows for the quantification of structural features for subtype selectivity in β-adrenergic ligands. Agonist-induced selectivity may be based on both differences in receptor-subtype binding and differences in the stimulation of adenylate cyclase coupled to both subtypes. For instance, the selectivity of salbutamol for β_2-adrenoceptors is predominantly due to its inability to stimulate the adenylate cyclase coupled to β_1-adrenoceptors [16]. To prevent this possible ambiguity we have investigated a number of compounds belonging to the class of the phenoxypropanolamines, usually antagonists, displaying little, if any, intrinsic activity. Their selectivity, thus, will merely reside in differences in receptor-subtype binding. In Table 4 the $-\log K_D$-values of 14 phenoxypropanolamine-derivatives are given, quantifying the interactions with β_2- (bovine m.trapezius) and β_1- (calf heart left ventricle) adrenoceptors respectively. For those compounds that are racemic mixtures the $-\log K_D$-values are raised with 0.3 (log 2), as only (−)-isomers are thought to be active on β-adrenoceptors [17]. No further corrections are needed, due to the ionization characteristics of these ligands, which are practically all monobasic acids (except prenalterol). Their pK_a-values range from 9.2 to 9.5 [18], the amounts of cations approximating 100% at pH = 7.5.

With the assumption that the influence of the *N*-isopropyloxypropanolamine side chain is identical for all compounds, we have attempted to correlate $-\log K_D$-values of the various substituted phenoxypropanolamines with calculated physico-chemical parameters of the corresponding substituted benzenes (Table 5). Using logP-values (octanol/water), calculated according to the hydrophobic fragmental system [8], only, we obtained the following regression equations by multiple regression analysis:

$$\beta_2: -\log K_D = 0.83(\pm 0.30)\log P_{calc} + 5.79(\pm 0.80) \quad (1)$$

$$n = 14 \; r = 0.6239 \; s = 0.9808 \; F = 7.647$$

$$\beta_1: -\log K_D = 0.64(\pm 0.17)\log P_{calc} + 6.23(\pm 0.46) \quad (2)$$

$$n = 14 \; r = 0.7328 \; s = 0.5658 \; F = 13.919$$

A considerable improvement of the equations was achieved after the introduction of calculated steric parameters. (Substitution at R_4 was not considered, due to single occurrence.) The use of the steric branching parameter (S_b) [9] was especially favoured, due to its easy calculation. This parameter largely accounts for branching of a substituent rather than for 'width' or 'length'. The equations are:

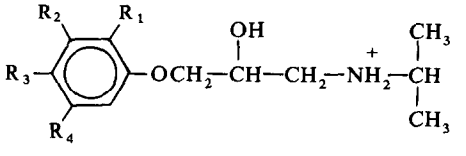
$$\beta_2: -\log K_D = 0.77(\pm 0.11)\log P_{calc} + 0.30(\pm 0.08)S_{b_{R_1}} - 0.25(\pm 0.11)S_{b_{R_2}} - 0.51(\pm 0.07)S_{b_{R_3}} + 6.03(\pm 0.27) \quad (3)$$

$$n = 14 \; r = 0.9757 \; s = 0.3176 \; F = 58.640$$

$$\beta_1: -\log K_D = 0.81(\pm 0.14)\log P_{calc} + 0.11(\pm 0.10)S_{b_{R_1}} - 0.35(\pm 0.13)S_{b_{R_2}} - 0.17(\pm 0.09)S_{b_{R_3}} + 5.96(\pm 0.34) \quad (4)$$

$$n = 14 \; r = 0.9126 \; s = 0.3925 \; F = 15.256.$$

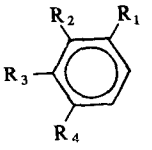
Table 4. $-\text{Log}K_D$-values of 14 phenoxypropanolamines interacting with β_2-adrenoceptors (bovine m. trapezius) and β_1-adrenoceptors (calf heart left ventricle). S.E. < 10% in K_D-values



Name	R_1	R_2	R_3	R_4	$-\text{Log}\,K_D$* for β_2	$-\text{Log}\,K_D$ for β_1
Propranolol	—CH=CH—CH=CH—		H	H	9.05	8.92
Alprenolol	CH_2CHCH_2	H	H	H	9.41	8.71
Practolol	H	H	$NHCOCH_3$	H	4.99	6.16
Kö 589	CH_3	H	H	H	8.29	8.13
Kö 707	H	CH_3	H	CH_3	8.29	8.08
Kö 1124	H	$CH_3CHCH_2CH_3$	H	H	8.05	7.80
Kö 1313	CN	H	H	H	7.96	7.88
Kö 1350	CH_2OH	H	H	H	7.60	7.29
Kö 592	H	CH_3	H	H	7.66	7.42
–	H	H	H	H	7.57	7.62
Kö 1411	OCH_2CCH	H	H	H	8.97	8.32
Prenalterol	H	H	OH	H	6.27	6.59
Betaxolol	H	H	$C_2H_4OCH_2$-cPr	H	7.03	8.37
Dihydro-alprenolol	$CH_2CH_2CH_3$	H	H	H	9.20	8.74

* Data from [11].

Table 5. Calculated physico-chemical parameters of substituted benzenes, corresponding to the aromatic nucleus of the phenoxypropanolamines



R_1	R_2	R_3	R_4	logP_{calc}	S_{bR_1}	S_{bR_2}	S_{bR_3}
—CH=CH—CH=CH—		H	H	3.295	1.0	1.0	0.0
CH_2CHCH_2	H	H	H	3.215	3.0	0.0	0.0
$CH_2CH_2CH_3$	H	H	H	3.579	3.0	0.0	0.0
H	H	$NHCOCH_3$	H	1.251	0.0	0.0	4.0
CH_3	H	H	H	2.541	1.0	0.0	0.0
H	CH_3	H	CH_3	3.060	0.0	1.0	0.0
H	$CH_3CHCH_2CH_3$	H	H	4.098	0.0	4.0	0.0
CN	H	H	H	1.666	2.0	0.0	0.0
CH_2OH	H	H	H	1.178	2.0	0.0	0.0
H	CH_3	H	H	2.541	0.0	1.0	0.0
H	H	H	H	2.022	0.0	0.0	0.0
OCH_2CCH	H	H	H	2.412	3.0	0.0	0.0
H	H	$C_2H_4OCH_2$-cPr	H	3.177	0.0	0.0	3.0
H	H	OH	H	1.526	0.0	0.0	1.0

The intercorrelation between log$P$ and the steric parameters is low, e.g. in equation (4) r for log$P$ vs S_{bR_1} is 0.07, vs S_{bR_2} 0.58 and vs S_{bR_3} −0.28. Of course, the use of four physico-chemical parameters with 14 derivatives only is somewhat questionable. The omission, however, of S_{bR_1}, leaving, statistically more acceptable, three independent variables, affects the equations only marginally (results not shown). The major conclusions to be drawn from equations (3) and (4) are:

the aromatic nuclei of the ligands interact with a rather lipophilic environment, which is virtually identical for both β_1- and β_2-adrenoceptors, as the regression coefficients for logP_{calc} are equal in both equations (0.77 vs 0.81).

the contribution of the aliphatic side chain to the affinity for both receptor-subtypes is identical, as the intercepts are equal (6.03 vs 5.96).

substitution at R_2, the position *meta* to the side chain is equally unfavourable in both equations, the regression coefficients being −0.25 and −0.35, respectively.

substitution at R_1, the position *ortho* to the side chain, seems to induce some β_2-selectivity (regression coefficient for β_2 is +0.30, for β_1 + 0.11), and appears to be largely sterically free.

β_1-selectivity is mainly achieved by substitution at R_3, the position *para* to the aliphatic side chain, rather by a sharp decrease in β_2-adrenoceptor affinity (steric hindrance) than an increase in β_1-adrenoceptor affinity (regression coefficient for β_2 being −0.51, for β_1 −0.17).

Some of these conclusions have been recognized as factors controlling affinity and selectivity previously. Coleman *et al.* have studied R_2/R_3-isomeric pairs of β-adrenoceptor antagonists, proving that substitution on R_3 within the pairs favours selectivity for rabbit heart membranes (β_1) compared to a membrane preparation of rat corpora lutea (β_2) [19]. Davies and coworkers have presented a qualitative model for *in vivo* β-adrenoceptors antagonistic action at cat cardiac sites of a series of *p*-acylamino derivatives, showing steric freedom for substituents on R_1 and R_3 [20, 21].

Finally, the β_1-selectivity of L643,717 is easily explained now: apart from the *N*-(3,4-dimethoxyphenyl)ethyl group, known to induce β_1-selectivity [22], the large thienylimidazole substituent, *para* to the oxypropanolamine side chain, is destructive for β_2-adrenoceptor affinity.

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