the irradiation of nitrogen gas by 16-MeV protons in the $^{14}N(p,\alpha)^{11}C$ reaction. After being trapped in a loop cooled by liquid nitrogen, the $^{11}CO_2$ was transferred by a stream of nitrogen into a cooled vessel (-78 °C) containing $LiAlH_4$ (10 μmol) in THF (500 μL). At the end of the trapping, the THF was removed under vacuum while heating to 130 °C. The vessel was once again cooled to -78 °C, and HI (1 mL, 54%) was added. Subsequent heating to 130 °C resulted in distillation of the $^{11}CH_3I$ formed. After passing through traps of soda lime and phosphorus pentoxide, the $^{11}CH_3I$ was trapped in 200 μL of Me_2SO. The amounts of activity obtained were dependent on the length of the irradiation of the target. A typical result was the preparation of 5.5 GBq (150 mCi) of [^{11}C]methyl iodide 7-8 min after end of bombardment (EOB) (30 μA, 10 min) with a specific activity of 11.1-18.5 GBq/mmol (300-500 Ci/mmol).

[^{11}C]-4-Isopropylantipyrine ([^{11}C]-1). The Me_2SO solution of [^{11}C]methyl iodide described above was transferred to a test tube containing **2** (1 mg, 0.0046 mmol), 100 μL of Me_2SO, and several grains of solid KOH. The mixture was stirred vigorously at 70 °C for 5 min. At the end of the reaction, 200 μL of the mobile phase for the HPLC was added and the resulting solution was injected onto the column (μ-Bondapak C-18, 300 × 7.8 mm, Waters). The mobile phase was a mixture of acetonitrile and H_3PO_4 (0.01 M) (35:65). A flow rate of 4 mL/min was used. The UV absorption at 241 nm and radioactivity were monitored simultaneously. The labeled [^{11}C]-1 eluted after 10.5 min, well-separated from labeled byproducts. The product was collected, evaporated to dryness in a rotary evaporator, and dissolved in a mixture of 3.5 mL of propylene glycol and 1.5 mL of ethanol. After addition of 5 mL of physiological saline and filtration through a Millipore filter (0.22 μm), a solution sterile and free from pyrogens was obtained: yield 40-50%; sp act. 3.7-7.4 GBq/mmol (100-200 Ci/mmol); time of preparation from EOB 40 min.

Animal Experiments. Male Wistar rats were used. The experiments were performed by the method described in Dahlgren et al.[15] for paralyzed and ventilated rats. Only a brief description of the main procedure will be given here. The animals were anesthetized in a jar using halothane (3.5%) in a mixture of oxygen and nitrous oxide (30:70). When unresponsive, they were tracheostomized and paralyzed (tubocurarine, iv 2 mg/kg), and then the halothane was decreased to 0.7% for the duration of the operative procedure which included placement of the necessary arterial and venous catheters. Blood pressure was over 100 mm Hg in all animals, P_{O_2} was adusted to surpass 100 mm Hg, P_{CO_2} was 36.9 ± 1.0 mmHg, and temperature was adjusted to close to 37 °C.

Details of the procedure used in the measurement of the partition coefficient are given in Abdul-Rahman et al.[12] In order to minimize the loss of the isotope during the experiment, the kidney blood vessels and ureters were ligated. Four animals received a bolus dose of [^{14}C]-1 (150 μCi/kg) at the start of the experiment and were then allowed an equilibration period of 45 min before decapitation. Blood samples taken during this period were frozen in liquid nitrogen. Radioactivity was then measured, in tissue and blood samples, using β-scintillation counting. The efficiency of the measurements was evaluated by adding internal standard to each sample ([^{14}C]hexadecane).

In the determination of CBF, [^{14}C]-1 was dissolved in Kreb's solution and infused at a steady rate over a period of 45 s (normocapnia) or 20 s (hypercapnia). During the infusion a set of timed blood samples were taken from the brachial artery, the last sample being taken at the time of decapitation. The brain was removed, frozen, and sectioned in 20-μm slices that were subsequently allowed to expose an X-ray film along with a set of calibrated standards for 1 week. The radioactivity was then assessed densitometrically by using an aperture of 1 mm (Macbeth TD 501). Calculation of the flow rates was performed by the method of Sakurada et al.[4]

In three animals CBF could not be calculated due to the precipitation of the tracer in the infusion solution. 4-Isopropylantipyrine is only slightly soluble in water. Since the specific activity of [^{14}C]-1 was so low (essentially the same at that of the [^{14}C]methyl iodide used in the labeling), dissolution was not complete in those cases in which the mass of the tracer used exceeded its solubility limit. This problem can be avoided in future studies by dissolution in a more lipophilic solution such as ethanol-propylene glycol-physiological saline (1.5:3.5:5). This solvent composition is often used for dissolution of lipophilic pharmaceuticals and is not known to affect cerebral blood flow in these low quantities.

Registry No. [^{14}C]-1, 96964-35-1; [^{11}C]-1, 96964-36-2; **2**, 50993-68-5; $^{14}CH_3I$, 16170-82-4; $^{11}CH_3I$, 54245-42-0; **3**, 89-25-8.

(15) Dahlgren, N.; Ingvar, M.; Siesjö, B. *J. Cerebral Blood Flow Metab.* **1981**, *1*, 429.

Quantitative Evaluation of the β_2-Adrenoceptor Affinity of Phenoxypropanolamines and Phenylethanolamines

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The influence of the aromatic moiety of β-adrenoceptor ligands on the affinity for the β_2-adrenoceptor has been studied. Three classes of ligands have been examined, viz. *N*-isopropyl- and N-*tert*-butylphenylethanolamines and *N*-isopropylphenoxypropanolamines. Computer-assisted analysis of the inhibition by any of these ligands of the specific (-)-[^{3}H]dihydroalprenolol binding to the β_2-adrenoceptors of a bovine skeletal muscle preparation in the presence of GppNHp (10^{-4} M) yielded the affinities of these ligands at pH 7.5. The obtained values were adjusted for the amounts of cations present at this pH value. A significant correlation was found between the calculated lipophilicities and the experimentally determined affinities in the three classes. Furthermore, steric factors seem to play an important role, as these correlations were improved by the introduction of steric parameters for the aromatic substituents in the regression analyses. From the established equations it is concluded that the phenoxypropanolamine derivatives bind to the β_2-adrenoceptor in a way different from that of the ligands in both ethanolamine classes.

The molecular basis of the interaction between the β-adrenoceptor and its ligands has been studied extensively.[1] The availability of radioactive ligands like [^{3}H]dihydroalprenolol (DHA), [^{125}I]hydroxybenzylpindolol (IHYP), and [^{125}I]cyanopindolol (ICYP) has been and still is an enormous impetus for the unraveling of triggering and regulatory steps in the hormone-receptor interaction. Surprisingly, qualitative considerations as well as quantitative analyses concerning the putative relation between physicochemical properties and biological activity (i.e., affinity and/or intrinsic activity) of β-adrenoceptor ligands

(1) A number of reviews have appeared, e.g.: Lefkowitz, R. J.; Caron, M. G.; Michel, T.; Stadel, J. M. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1982**, *41*, 2664. Also: Caron, M. G.; Shorr, R. G. L.; Lavin, T. N.; Lefkowitz, R. J. *Metabolism* **1982**, *31*, 658.

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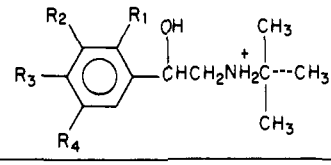
Table I. β_2-Adrenoceptor Affinities of Substituted *N-tert*-Butylphenylethanolamines



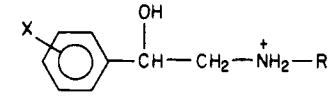
no.	compd	R_1	R_2	R_3	R_4	app K_D,[a] μM	cations present at pH 7.5[b]	isomer	cor[c] −log K_D	calcd −log K_D (acc to eq 17)
1	Th 1206	H	OH	OH	H	0.60	93	±	6.55	6.37
2	terbutaline	H	OH	H	OH	3.98	93	±	5.73	5.96
3	*N-tert*-butylnorsynephrine	H	H	OH	H	1.26	100	±	6.20	6.45
4	Du 28663	H	NH_2	OH	H	2.04	100	±	5.99	6.05
5	SKF 56301	H	$NHCH_3$	OH	H	0.52	100	±	6.58	6.64
6	salbutamol	H	CH_2OH	OH	H	0.52	100	±	6.58	6.39
7	AH 3474	H	$CONH_2$	OH	H	0.47	40	±	7.03	6.87
8	clenbuterol	H	Cl	NH_2	Cl	0.036	100	±	7.74	7.66
9	C78	Cl	H	H	H	0.13	100	±	7.18	6.97
10	VUF 8303	H	Cl	OH	Cl	1.95	4	±	7.41	7.63

[a] K_D values are the means of three to five separate experiments performed in duplicate, the standard error of the geometric mean in all cases being less than 10%. [b] As derived from the macroscopic ionization constants (see text). [c] Corrected for the amount of cations present at pH 7.5 and for isomerism: K_D for (−)-isomer = $0.5K_D$ for racemate and −log K_D for (−)-isomer = −log K_D for racemate + 0.30.

have been mainly based on results of pharmacological studies both in vivo and on isolated organs.[2] In these types of studies a number of phenomena can obscure the mere interaction between ligand and receptor, and it is unlikely that established correlations always will lead to unambiguous predictions. Among these complicating factors are receptor reserve, i.e. differences in the efficiency of β-adrenoceptor agonists, tissue selectivity or specificity, and, extended to in vivo circumstances, pharmacokinetics and metabolism.

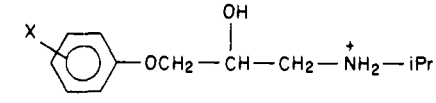
A limited number of structure–activity studies, however, deal with results obtained from experiments with isolated, fragmented cell membranes. Dunn et al.[3] have been able to distinguish between agonists and antagonists applying the SIMCA method of pattern recognition. This differentiation has been derived from data for a series of phenylethylamine agonists and antagonists of the frog erythrocyte β-adrenoceptor, obtained by Mukherjee et al.[4] Unfortunately, in this study intrinsic activities are probably underestimated, as GTP—or its stable analogue GppNHp, a necessary cofactor in the cascade leading from occupation of the receptor to the production of cAMP, the "second messenger"—was not included in the assay.[5] Šolmajer et al.[6] have examined the effects of the aliphatic side chain of phenylethylamine derivatives on β-adrenoceptor affinity, showing a correlation between affinity and approximate electrostatic potentials near the amino function. This latter study was based on data obtained with the turkey erythrocyte β-adrenoceptor in the presence of GppNHp.[7]

Recently, we have shown that among the four ionic species of β-adrenoceptor ligands possible at physiological pH (cation, zwitterion, uncharged molecule, anion) the cation governs affinity.[8] This feature has been established by the pH-dependent specific displacement of the radioligand DHA by some β-adrenoceptor ligands from a membrane preparation containing mammalian β-adrenoceptors. Extending these studies we have now focused on the contribution to β-adrenoceptor affinity of the aromatic moiety present in the three main classes of β-adrenoceptor ligands, viz. the *N*-isopropyl- and *N-tert*-butylphenylethanolamines (A) and the *N*-isopropylphenoxypropanolamines (B).



R = iPr or tBu

A



B

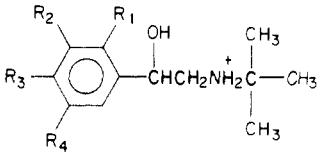
−log K_D values, obtained from radioligand binding studies, have been used as a measure for the affinity of the ligands. As will be shown in this paper, the affinities of the ligands for the receptor can be mathematically described by a combination of various calculated physicochemical parameters.

Results

The K_D values of derivatives within the two classes of phenylethanolamines and the class of the phenoxypropanolamines are listed in Tables I–III, respectively. As has been mentioned, the cationic species of the ligands governs β_2-adrenoceptor affinity.[8] Thus, all experimentally determined K_D values have been adjusted for the amounts of cations present at pH 7.5, which especially is important in the phenylethanolamines, usually dibasic acids. The fraction of cations is easily derived from the macroscopic ionization constants of the compounds.[9] Moreover, in case of the racemates, −log K_D values have been raised with 0.3 (log 2), as only (−)-isomers are considered contributing to

(2) For excellent and comprehensive reviews, see: Topliss, J. G. "Medicinal Chemistry: Quantitative Structure-Activity Relationships of Drugs"; Academic Press: New York, 1983; Vol. 19, Chapters 5 and 6.
(3) Dunn, W. J.; Wold, S.; Martin, Y. C. *J. Med. Chem.* **1978**, *21*, 922.
(4) Mukherjee, C.; Caron, M. C.; Mulliken, D.; Lefkowitz, R. J. *Mol. Pharmacol.* **1976**, *12*, 16.
(5) This has been shown earlier by: Bilezikian, J. P. "Hormone-Receptors"; Levey, G., Ed.; Academic Press: New York, 1976; p 355.
(6) Šolmajer, T.; Lukovits, I.; Hadži, D. *J. Med. Chem.* **1982**, *25*, 1413.
(7) Bilezikian, J. P.; Dornfeld, A. M.; Gammon, D. E. *Biochem. Pharmacol.* **1978**, *27*, 1445.
(8) IJzerman, A. P.; Bultsma, T.; Timmerman, H.; Zaagsma, J. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1984**, *327*, 293.
(9) For method see: IJzerman, A. P.; Bultsma, T.; Timmerman, H.; Zaagsma, J. *J. Pharm. Pharmacol.* **1984**, *36*, 11.

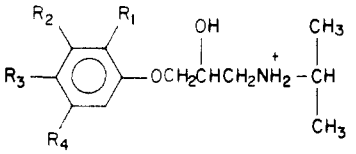
Table II. β_2-Adrenoceptor Affinities of Substituted *N*-Isopropylphenylethanolamines



no.	compd	R_1	R_2	R_3	R_4	app K_D,[a] μM	cations present at pH 7.5[b]	isomer	cor[c] $-\log K_D$	calcd $-\log K_D$ (acc to eq 17)
11	isoprenaline	H	OH	OH	H	0.33	93	-	6.51	5.92
12	orciprenaline	H	OH	H	OH	9.12	93	±	5.37	5.51
13	Du 21117	H	NH_2	OH	H	5.13	100	±	5.59	5.60
14	AH 3021	H	CH_2OH	OH	H	3.16	100	±	5.80	5.93
15	NAB 277	H	Cl	NH_2	Cl	0.058	100	±	7.54	7.21
16	*N*-isopropylnorsynephrine	H	H	OH	H	4.27	100	±	5.67	5.99
17	pronethalol	H	*d*	*d*	H	0.16	100	±	7.10	7.30
18	INPEA	H	H	NO_2	H	1.32	100	±	6.18	6.61
19	sotalol	H	H	$NHSO_2CH_3$	H	0.58	92	±	6.57	6.60
20	*N*-isopropylnorphenylephrine	H	OH	H	H	0.91	100	±	6.34	5.99

[a] K_D values are the means of three to five separate experiments performed in duplicate, the standard error of the geometric mean in all cases being less than 10%. [b] As derived from the macroscopic ionization constants (see text). [c] Corrected for the amount of cations present at pH 7.5 and for isomerism: K_D for (-)-isomer = $0.5K_D$ for racemate and $-\log K_D$ for (-)-isomer = $-\log K_D$ for racemate + 0.30. [d] -CH=CH—CH=CH-.

Table III. β_2-Adrenoceptor Affinities of Substituted *N*-Isopropylphenoxypropanolamines



no.	compd	R_1	R_2	R_3	R_4	app K_D,[a] nM	cations present at pH 7.5[b]	isomer	cor[c] $-\log K_D$	calcd $-\log K_D$ (acc to eq 16)
21	propranolol	*f*	*f*	H	H	0.89	100	-	9.05	8.63
22	alprenolol	CH_2CHCH_2	H	H	H	0.39	100	-	9.41	9.44
23	dihydroalprenolol[d]	$CH_2CH_2CH_3$	H	H	H	0.63	100	-	9.20	9.79
24	practolol	H	H	$NHCOCH_3$	H	20417	100	±	4.99	5.17
25	Kö 589	CH_3	H	H	H	10.23	100	±	8.29	8.34
26	Kö 707	H	CH_3	H	CH_3	10.23	100	±	8.29	8.18
27	Kö 1124	H	$CH_3CHC_2H_5$	H	H	17.78	100	±	8.05	7.90
28	Kö 1313	CN	H	H	H	21.88	100	±	7.96	7.72
29	Kö 1350	CH_2OH	H	H	H	50.12	100	±	7.60	7.26
30	Kö 592	H	CH_3	H	H	43.65	100	±	7.66	7.68
31		H	H	H	H	53.70	100	±	7.57	7.61
32	Kö 1411	OCH_2CCH	H	H	H	2.14	100	±	8.97	8.89
33	prenalterol	H	H	OH	H	1072	100	±	6.27	6.71
34	betaxolol	H	H	$C_2H_4OCH_2cPr$[e]	H	186.21	100	±	7.03	7.02

[a] K_D values are the means of three to five separate experiments performed in duplicate, the standard error of the geometric mean in all cases being less than 10%. [b] As derived from the macroscopic ionization constants (see text). [c] Corrected for the amount of cations present at pH 7.5 and for isomerism: K_D for (-)-isomer = $0.5K_D$ for racemate and $-\log K_D$ for (-)-isomer = $-\log K_D$ for racemate + 0.30. [d] This compound is tritiated alprenolol. [e] cPr = cyclopropyl. [f] -CH=CH—CH=CH-.

activity on β-adrenoceptors.[10]

If one assumes the influence of the aliphatic side chain on β-adrenoceptor affinity to be identical within one class, it is permissible to relate physicochemical properties of the aromatic moieties with the affinities of the whole molecules. Thus, by considering the β-adrenoceptor ligands as the corresponding substituted benzenes, we easily obtained a large and diverse parameter data set. This would have been far more difficult or even impossible with respect to the "intact" derivatives, apart from the fact that measurement of the hydrophobicity of charged molecules (i.e. cations), especially the hydrophilic phenylethanolamines, is rather cumbersome. Examining the various parameters in detail, we have considered the lipophilic (hydrophobic) characteristics of the substituted benzenes first. Although experimentally determined log *P* values (octanol/water) are available for a large number of benzenes,[12] the listing is not complete (see Table IV). Therefore, we have calculated the log *P* values of all compounds, according to the hydrophobic fragmental system, as described by Rekker and De Kort.[11] A close correlation between calculated and experimentally determined log *P* values was observed (eq 1), placing reliance on the other calculated values. For 47

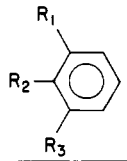
$$\log P_{calcd} = 0.97\ (\pm 0.02)\ \log P_{exptl} + 0.04\ (\pm 0.05) \qquad (1)$$

$$n = 17,\ r = 0.9957,\ s = 0.0970,\ F = 1721.68$$

we have used the experimentally determined value (0.95), as for this sulfoanilide no reliable estimation is available. With respect to steric parameters an analogous reasoning

(10) See for instance: Patil, P. N.; Miller, D. D.; Trendelenburg, U. *Pharmacol. Rev.* **1975**, *26*, 323.
(11) Rekker, R. F.; De Kort, H. M. *Eur. J. Med. Chem.* **1979**, *14*, 479.
(12) Hansch, C.; Leo, A. "Substituent Constants for Correlation Analysis in Chemistry and Biology"; Wiley: New York, 1979.
(13) Austel, V.; Kutter, E.; Kalbfleisch, W. *Arzneim.-Forsch./Drug Res.* **1979**, *29*, 585.

Table IV. Calculated[a] and Experimentally Determined[b] Partition Coefficients (Octanol/Water) of Substituted Benzenes



no.	R_1	R_2	R_3	log P_{calcd}	log P_{exptl}
35	H	H	H	2.022	2.13
36	OH	OH	H	1.030	0.88
37	OH	H	OH	0.741	0.80
38	OH	H	H	1.526	1.46
39	$NHCH_3$	OH	H	1.098	
40	CH_2OH	OH	H	0.682	0.73
41	$CONH_2$	OH	H	1.103	1.28
42	Cl	OH	Cl	3.010	
43	Cl	H	H	2.764	2.84
44	Cl	NH_2	Cl	3.060	
45	c	c	H	3.295	3.30
46	NO_2	H	H	1.787	1.85
47	$NHSO_2CH_3$	H	H		0.95
48	CH_2CHCH_2	H	H	3.215	3.23
49	$CH_2CH_2CH_3$	H	H	3.579	3.57
50	$NHCOCH_3$	H	H	1.251	1.16
51	CH_3	H	H	2.541	2.73
52	CH_3	H	CH_3	3.060	3.20
53	$CH_3CHC_2H_5$	H	H	4.098	
54	CN	H	H	1.666	1.56
55	CH_2OH	H	H	1.178	1.10
56	NH_2	OH	H	0.502	0.52
57	OCH_2CCH	H	H	2.412	
58	$C_2H_4OCH_2cPr$	H	H	3.177	

[a] Calculation according to ref 11. [b] All values are taken from ref 12. [c] -CH=CH—CH=CH-.

can be followed: Taft's E_s is not available for all substituents that occur in Table IV, neither is Charton's ν.[14] Verloop's L, B_1, B_2, B_3, and B_4 are parameters, which can be calculated, but for a reasonable definition of spatial characteristics often more than one parameter is needed.[15] Furthermore, the calculation of these five parameters has been matter of debate.[16] Bearing this in mind, we were prompted to develop a steric measure for the substituents in our data set. A computer program developed by one of us and designed for the calculation of several volume and area parameters was used.[17] In Table V ΔSA and ΔVL for the substituents are listed, which represent the differences in "ordinary" (i.e., nonsmoothed) volume and surface area, respectively, between the substituted benzene and the C_6H_5 fragment. A close correlation is observed between ΔSA and ΔVL (eq 2). Furthermore, the easily

$$\Delta SA = 1.72\ (\pm 0.12)\ \Delta VL - 0.64\ (\pm 3.89) \quad (2)$$

$$n = 18,\ r = 0.9651,\ s = 7.7374,\ F = 217.053$$

accessible Sb (steric branching) parameter, introduced by Austel et al., was calculated and tabulated for all substituents.[13] Sb also shows a correlation with ΔSA and ΔVL, although to a lesser extent, reflecting the somewhat distinct character of this parameter (eq 3). In a first attempt we

$$\Delta VL = 8.82\ (\pm 2.15)\ Sb + 8.18\ (\pm 5.86) \quad (3)$$

$$n = 18,\ r = 0.7153,\ s = 11.5641,\ F = 16.763$$

have correlated $-\log K_D$ with log P_{calcd} (eq 4–6) (t-Bu = t-Bu: $-\log K_D = 0.53\ (\pm 0.12)\ \log P_{calcd} + 5.87\ (\pm 0.23)$ (4)

$$n = 10,\ r = 0.8353,\ s = 0.3724,\ F = 18.471$$

i-Pr: $-\log K_D = 0.58\ (\pm 0.15)\ \log P_{calcd} + 5.40\ (\pm 0.26)$ (5)

$$n = 10,\ r = 0.8089,\ s = 0.4318,\ F = 15.144$$

oxy: $-\log K_D = 0.83\ (\pm 0.30)\ \log P_{calcd} + 5.79\ (\pm 0.80)$ (6)

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

N-$tert$-butylphenylethanolamines, i-Pr = N-isopropylphenylethanolamines, oxy = N-isopropylphenoxypropanolamines). The equations for t-Bu and i-Pr can be combined with the introduction of a dummy parameter ($D = 1$ for t-Bu, $D = 0$ for i-Pr), somehow representing the extra methylene group in t-Bu (eq 7). This equation

t-Bu/i-Pr: $-\log K_D = 0.55\ (\pm 0.09)\ \log P_{calcd} + 0.41\ (\pm 0.18)\ D + 5.43\ (\pm 0.19)$ (7)

$$n = 20,\ r = 0.8313,\ s = 0.4026,\ F = 20.549$$

accounts for the higher affinity of the t-Bu derivatives, a well-known phenomenon in in vitro studies with isolated organs.[18] Although, in the class of the phenylethanolamines, the contribution of lipophilicity to binding explains more than 50% of the observed variance, the regression equations could be considerably improved by the introduction of steric parameters. Other parameters such as Hammett's σ and Kier's χ were used too, alone or in combination with log P_{calcd}, but proved to be unsuccessful (equations not shown).[19] As the regression coefficients

Table V. Steric Substituent Parameters

substituent	ΔSA[a]	ΔVL[b]	Sb[c]	E_s
-H	8.4	3.1	0.0	0.00
$-CH_3$	29.9	14.2	1.0	-1.24
n-C_3H_7	73.0	36.5	3.0	-1.60
$-CH_2CH{=}CH_2$	52.5	35.8	3.0	
$-CH(CH_3)C_2H_5$	93.8	47.6	4.0	-2.37
$-CH_2OH$	37.7	21.0	2.0	-1.21
$-C_2H_4OCH_2cPr$	129.9	69.9	3.0	
-CN	34.9	23.6	2.0	-0.51
$-CONH_2$	45.2	27.2	3.0	
$-NH_2$	23.8	12.4	1.0	-0.61
$-NHCH_3$	45.1	23.4	2.0	
$-NHCOCH_3$	50.7	33.4	4.0	
$-NHSO_2CH_3$	60.0	42.4	5.2	
$-NO_2$	38.1	23.0	3.0	-2.52
-OH	19.9	10.4	1.0	-0.55
$-OCH_2C{\equiv}CH$	67.2	42.7	3.0	-1.89 (?)
-Cl	31.6	21.9	1.2	-0.97
-CH=CH—CH=CH-	59.2	41.3	2.0[d]	

[a] ΔSA in Å^2. [b] ΔVL in Å^3. [c] Sb steric branching parameter.[13] [d] Treated as 2 × 1.0 in case of propranolol.

(14) For E_s, see e.g.: Taft, R. W. *J. Am. Chem. Soc.* **1952**, *74*, 3120. For E_s values (E_s for -H = 0), see: Unger, S. H.; Hansch, C. *Prog. Phys. Org. Chem.* **1976**, *12*, 91. For ν values, see e.g.: Charton, M. *J. Am. Chem. Soc.* **1975**, *97*, 1552.

(15) See for instance: Verloop, A.; Tipker, J. *Pestic. Sci.* **1976**, *7*, 379. Also: Verloop, A.; Hoogenstraten, W.; Tipker, J. "Drug Design"; Ariëns, E. J., Ed.; Academic Press: New York, 1976; Vol. 7, p 165.

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(17) Bultsma, T. *Eur. J. Med. Chem.* **1980**, *15*, 371. The definition of coordinates is in accordance with the instructions in this paper.

(18) Mylecharane, E. J.; Raper, C. *Eur. J. Pharmacol.* **1973**, *21*, 375.

(19) **Hammett** σ. We have used σ_m and σ_p that are given by ref 12, in combination with log P_{calcd}. **Kier** χ. We have used χ^v values only (without including log P_{calcd}), according to: Kier, L. B.; Hall, L. H. "Molecular Connectivity in Chemistry and Drug Research"; Academic Press: New York, 1976.

for the substituents R_2 and R_3 (*meta* and *para* to the aliphatic side chain, respectively) appeared to be approximately identical within one class, we have summed the respective parameter values and used these in eq 8–16.

t-Bu: $-\log K_D = 0.57\ (\pm 0.08) \log P_{calcd} + 0.017\ (\pm 0.005)\ \Delta SA_{R_2+R_3} + 5.05\ (\pm 0.28)$ (8)

$n = 10,\ r = 0.9357,\ s = 0.2555,\ F = 28.134$

$-\log K_D = 0.53\ (\pm 0.08) \log P_{calcd} + 0.025\ (\pm 0.008)\ \Delta VL_{R_2+R_3} + 5.25\ (\pm 0.24)$ (9)

$n = 10,\ r = 0.9322,\ s = 0.2620,\ F = 26.573$

$-\log K_D = 0.63\ (\pm 0.07) \log P_{calcd} + 0.27\ (\pm 0.06)\ Sb_{R_2+R_3} + 5.17\ (\pm 0.20)$ (10)

$n = 10,\ r = 0.9543,\ s = 0.2164,\ F = 40.615$

i-Pr: $-\log K_D = 0.55\ (\pm 0.14) \log P_{calcd} + 0.014\ (\pm 0.010)\ \Delta SA_{R_2+R_3} + 4.80\ (\pm 0.47)$ (11)

$n = 10,\ r = 0.8403,\ s = 0.4257,\ F = 9.896$

$-\log K_D = 0.53\ (\pm 0.15) \log P_{calcd} + 0.017\ (\pm 0.012)\ \Delta VL_{R_2+R_3} + 5.05\ (\pm 0.36)$ (12)

$n = 10,\ r = 0.8300,\ s = 0.4379,\ F = 9.158$

$-\log K_D = 0.61\ (\pm 0.13) \log P_{calcd} + 0.20\ (\pm 0.10)\ Sb_{R_2+R_3} + 4.91\ (\pm 0.32)$ (13)

$n = 10,\ r = 0.8694,\ s = 0.3880,\ F = 12.628$

oxy: $-\log K_D = 1.02\ (\pm 0.19) \log P_{calcd} + 0.016\ (\pm 0.008)\ \Delta SA_{R_1} - 0.016\ (\pm 0.006)\ \Delta SA_{R_2+R_3} + 5.51\ (\pm 0.45)$ (14)

$n = 14,\ r = 0.9091,\ s = 0.5727,\ F = 19.369$

$-\log K_D = 0.98\ (\pm 0.16) \log P_{calcd} + 0.029\ (\pm 0.011)\ \Delta VL_{R_1} - 0.028\ (\pm 0.008)\ \Delta VL_{R_2+R_3} + 5.49\ (\pm 0.39)$ (15)

$n = 14,\ r = 0.9331,\ s = 0.4942,\ F = 27.148$

$-\log K_D = 0.94\ (\pm 0.13) \log P_{calcd} + 0.32\ (\pm 0.11)\ Sb_{R_1} - 0.28\ (\pm 0.07)\ Sb_{R_2+R_3} + 5.52\ (\pm 0.34)$ (16)

$n = 14,\ r = 0.9477,\ s = 0.4387,\ F = 35.347$

Again, the equations for t-Bu and i-Pr can be combined with the same dummy parameter (results are only shown for Sb, eq 17). Steric parameters for R_1 for the phenyl-

t-Bu/i-Pr: $-\log K_D = 0.61\ (\pm 0.07) \log P_{calcd} + 0.23\ (\pm 0.06)\ Sb_{R_2+R_3} + 0.45\ (\pm 0.13)\ D - 4.83\ (\pm 0.20)$ (17)

$n = 20,\ r = 0.9173,\ s = 0.2974,\ F = 32.250$

ethanolamines and for R_4 for all three classes were not included, since only few substituents are in the data set for these positions. The intercorrelations between log P_{calcd} and the various steric parameters are low. In eq 16, for instance, the correlation coefficients for log P_{calcd} vs. Sb_{R_1} or $Sb_{R_2+R_3}$ are 0.07 and 0.23, respectively. The difference in the regression coefficients for log P_{calcd} in eq 16 and 17 is statistically significant.[28] The 90% confidence limits are 0.24 and 0.12, respectively, with no obvious overlap in both regression coefficients.

Discussion

Careful analysis of the derived equations gives rise to the following conclusions:

log P_{calcd} is an important parameter in β_2-adrenoceptor affinity (eq 5–17). The regression coefficients for log P_{calcd} in the two classes of phenylethanolamines are distinct from the one in the class of the phenoxypropanolamines (eq 7 vs. eq 6, and eq 14–16 vs. eq 17); i.e., the contribution of lipophilicity to the affinity of the phenoxypropanolamines represents a larger part of the total affinity than in the case of the phenylethanolamines. It has recently been shown that lipophilicity is the only parameter determining the so-called nonspecific (but displaceable) binding of β-adrenoceptor ligands.[20] On the basis of our results it may be concluded that lipophilicity is important in specific receptor binding as well.

From the equations in which the steric parameters are included (eq 8–17), it emerges that Sb, the parameter that accounts for branching of substituents, appears to be more suitable than ΔSA and ΔVL, the measures for $X_{1,2}$ surface and X_1 volume, respectively. Correlation coefficients (and the other statistics) tend to be better in all cases, if Sb is used in the description of the steric requirements for this ligand–receptor interaction. Although Sb is a somewhat intuitively developed parameter, its application is favored by the easy calculation for any conceivable substituent.

Clearly, the steric requirements for the phenoxypropanolamines are in contrast with those for both classes of phenylethanolamines. Substitution of H by R_2 and R_3 is unfavorable in the phenoxypropanolamines, whereas this substitution pattern is advantageous in the phenylethanolamines. Ortho (R_1) substitution does not seem to be sterically limited in the phenoxypropanolamines. It enhances β_2-adrenoceptor affinity, due to the introduction of both extra lipophilicity and steric "bulk" in the compounds. Since only one ortho substituent is found in the phenylethanolamines (compound **9**), the importance of this position still remains unclear. As has been mentioned before, the same holds true for R_4. The number of substituents, especially in the class of the *N-tert*-butylphenylethanolamines, is rather limited, due to the rationales that have been used for the design of β-adrenoceptor agonists (often a phenolic OH group present). The close resemblance between the regression equations for the *N*-isopropyl- and *N-tert*-butylphenylethanolamines, however, represents, in our opinion, evidence that the performed analysis describes the interaction between the β-adrenoceptor and the *N-tert*-butyl derivatives fairly well.

A number of conformational studies on β-adrenoceptor ligands have been performed. X-ray diffraction,[21] proton NMR, predominantly in $CDCl_3$,[22,23] and IR spectroscopy[23] were among the experimental methods, whereas in quantum-mechanical studies computer-assisted calculation methods like PCILO[24] and SCF–MO–LCAO[25] were used. Although all methods almost exclusively deal with the ligands only (either in a nonphysiological solution, in the crystalline state, or in the gas phase), most authors speculate on the preferred conformation of these ligands on the β-adrenoceptor. Thus, Jen and Kaiser have proposed a "rigid" bicyclic structure for the phenoxypropanolamines, which can be superimposed on the phenylethanolamines in a way that both phenyl rings are in the same position.[22]

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This feature has been corroborated in a recent MO study.[25] On the other hand, Zaagsma suggested a counteranion—or anionic group—playing an important role in the conformation of phenoxypropanolamines, leading to a seven-membered ring structure, using essentially the same technique as Jen and Kaiser.[23]

From our regression analyses (eq 16 and 17) we conclude that the phenyl nucleus of the phenoxypropanolamines is in a position different from the phenyl nucleus of the phenylethanolamines, for two reasons. First, the regression coefficient for log P_{calcd} is significantly higher in the class of the phenoxypropanolamines. This feature suggests a more lipophilic environment for the ring structure, e.g. a region rich of tryptophan moieties, as proposed for propranolol.[26] Second, the steric requirements with respect to R_2 and R_3 are different for both classes, suggesting a different spatial environment as well. Thus, our results are contradictory with the hypothesis—the bicyclic structure—of Jen and Kaiser[22] but are not incompatible, however, with a structure as proposed by Zaagsma.[23] Bearing this in mind, we can speculate on the influence of the "side chain". From the intercepts in eq 16 and 17 (5.52 for the phenoxypropanolamines and 4.83 for the corresponding *N*-isopropylphenylethanolamines) the relative importance of the side chain for β_2-adrenoceptor affinity clearly emerges. This, of course, has to be predominantly due to the combined interaction of the protonated amino function and the β-hydroxy group with the receptor. Lengthening of the side chain through the insertion of an extra $-OCH_2-$ "bridge", as in the phenoxypropanolamines, raises the affinity of the side chain, suggesting a certain interaction of this bridge with the β-adrenoceptor. Furthermore, this ether linkage permits the phenyl nucleus "stacking" in a more hydrophobic region, contributive to a higher affinity, too.

The effects of the side chain have been examined by Šolmayer et al. as well.[6] These authors have found a correlation between the affinity toward β-adrenoceptors (K_D values from the study of Bilezikian)[7] of phenylethylamine derivatives, using approximated electrostatic potentials. Unfortunately, the amino function was supposed to be uncharged, and it was concluded that the electrostatic potential in the region of the :N lone-pair electrons is important for the binding at the β-adrenoceptor. As the cation of β-adrenoceptor ligands governs β-adrenoceptor affinity, the above conclusion has to be seriously questioned, however.

Conclusions

Radioligand binding studies, as a direct measure of ligand–receptor interactions combined with a conventional QSAR analysis are a valuable tool in the elucidation of the nature of these interactions. With respect to the β-adrenoceptor it is concluded that the orientation and/or position of the phenyl nucleus in the phenoxypropanolamines is different from both classes of phenylethanolamines, suggesting the ethanolamine fragment $[-CH(OH)-CH_2NH_2^+-]$ is superimposed in all three classes.

Experimental Section

β_2-Adrenoceptor Affinity. K_D values (as a measure for β-adrenoceptor affinity) were determined by a computer-assisted analysis of the inhibition of the specific (−)-[^{3}H]dihydroalprenolol (DHA) binding at pH 7.5 to the β_2-adrenoceptors of a bovine skeletal muscle preparation. This preparation is a suspension of the final, washed 40000*g* pellet, obtained by differential centrifugation of a homogenate of the musculus trapezius. Details of the method employed, concerning data analysis, preparation of the membranes, and [^{3}H]DHA binding assay, have been previously described.[8] K_D values were adjusted as described in the Results.

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,[11] as described in the Results.

Steric Parameters. The steric branching parameter (Sb) for all substituents was calculated according to Austel et al.[13] Furthermore, the differences in ordinary surface areas or volumes (ΔSA and ΔVL, respectively) between the C_6H_5 fragment and (substituted) benzenes were calculated with a computer program written by Bultsma,[17] originally designed for the estimation of geometric parameters of intact molecules. In this way, nonsmoothed steric parameters for all substituents were obtained.

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

Chemistry. The synthesis of all compounds listed has been published, except for VUF 8303 (**10**), whose synthesis is described below. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained on a Bruker WH-90 spectrometer and mass spectra on a Finnigan 4000 GC/MS spectrometer (70 eV). Spectral data were in accordance with the assigned structure. Melting points were determined on a Mettler FP5 melting point apparatus. Elemental analyses were performed by TNO, Zeist, The Netherlands, and values are within 0.4% of theory. Chemical purity was checked by titration with aqueous KOH (0.1 M).

***N*-*tert*-Butyl-2-(3,5-dichloro-4-hydroxyphenyl)-2-hydroxyethylamine Hydrogen Chloride (10), VUF 8303.** A 1.05-g (5 mmol) portion of **3** (free base) was dissolved in 10 mL of HCl (4 M). Chlorine was introduced into the solution until a gain of weight of 0.9 g and a white precipitate formed, the remaining solution being yellow. After suction and washing with HCl (4 M) and Et_2O, the product was crystallized from EtAc/MeOH (30/70, v/v); mp 229–230 °C. Anal. ($C_{12}H_{17}Cl_2NO_2$·HCl) C, H, N. Titration: 100.2%. M^+ (free base): found, 277.0642; calcd, 277.0636.

Materials. The following compounds were gifts: orciprenaline and Th 1206 (sulfates, Boehringer Ingelheim), terbutaline (sulfate, Astra), AH 3021 and salbutamol (bases, Allenburys), AH 3474 (hydrochloride, Allenburys), *N*-*tert*-butylnorsynephrine (base, Duphar), Du 21117 and Du 28663 (sulfates, Duphar), *N*-isopropylnorphenylephrine (hydrochloride, SKF), *N*-isopropylnorsynephrine (D-tartrate, SKF), SKF 56301 (base, SKF), clenbuterol and NAB 277 (hydrochlorides, Karl Thomae), C78 (hydrochloride, UCB), sotalol (hydrochloride, Mead Johnson), INPEA (hydrochloride, Selvi), practolol (base, ICI), pronethalol (hydrochloride, ICI), betaxolol (hydrochloride, Synthélabo), alprenolol and prenalterol (hydrochlorides, Hässle), all Kö compounds (hydrochlorides, Boehringer Ingelheim). Compound **31** has been published before.[27] Isoprenaline and propranolol (hydrochlorides) were obtained from Sigma. Dihydroalprenolol, tritiated, was purchased from Amersham (sp act. 70 Ci/mmol). All other reagents were of analytical grade.

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Registry No. 1, 18866-78-9; **2**, 46719-29-3; **3**, 96948-64-0; **4**, 96948-65-1; **5**, 96948-66-2; **6**, 35763-26-9; **7**, 27107-74-0; **8**, 50306-01-9; **9**, 73575-60-7; **10**, 96948-67-3; **10**·HCl, 93739-47-0; **11**, 51-31-0; **12**, 13392-25-1; **13**, 96948-68-4; **14**, 76166-84-2; **15**, 50306-04-2; **16**, 3506-31-8; **17**, 2238-85-9; **18**, 7413-36-7; **19**, 27948-47-6; **20**, 10104-00-4; **21**, 4199-09-1; **22**, 23846-71-1; **23**, 96948-69-5; **24**, 23313-50-0; **25**, 67834-82-6; **26**, 34441-05-9; **27**, 20235-53-4; **28**, 96948-70-8; **29**, 96948-71-9; **30**, 20917-89-9; **31**, 66527-95-5; **32**, 96948-72-0; **33**, 62340-37-8; **34**, 87896-30-8; **35**, 71-43-2; **36**, 120-80-9; **37**, 108-46-3; **38**, 108-95-2; **39**, 611-24-5; **40**,

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90-01-7; 41, 65-45-2; 42, 87-65-0; 43, 108-90-7; 44, 608-31-1; 45, 91-20-3; 46, 98-95-3; 47, 1197-22-4; 48, 300-57-2; 49, 103-65-1; 50, 103-84-4; 51, 108-88-3; 52, 108-38-3; 53, 135-98-8; 54, 100-47-0; 55, 100-51-6; 56, 95-55-6; 57, 13610-02-1; 58, 96948-73-1.

Supplementary Material Available: Tables analogous to Tables I–III with the various physicochemical parameters included (3 pages). Ordering information is given on any current masthead page.

Functionalized Congeners of 1,3-Dialkylxanthines: Preparation of Analogues with High Affinity for Adenosine Receptors

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A series of functionalized congeners of 1,3-dialkylxanthines has been prepared as adenosine receptor antagonists. On the basis of the high potency of 8-(*p*-hydroxyphenyl)-1,3-dialkylxanthines, the parent compounds were 8-[4-[(carboxymethyl)oxy]phenyl] derivatives of theophylline and 1,3-dipropylxanthine. A series of analogues including esters of ethanol and *N*-hydroxysuccinimide, amides, a hydrazide, an acylurea, and anilides were prepared. The potency in blocking A_1-adenosine receptors (inhibition of binding of N^6-[^{3}H]cyclohexyladenosine to brain membranes) and A_2-adenosine receptors (inhibition of 2-chloroadenosine-elicited accumulations of cyclic AMP in brain slices) was markedly affected by structural changes distal to the primary pharmacophore (8-phenyl-1,3-dialkylxanthine). Potencies in the dipropyl series at the A_1 receptor ranged from K_i values of 1.2 nM for a congener with a terminal amidoethyleneamine moiety to a K_i value of 58 nM for the parent carboxylic acid to a K_i of 96 nM for the bulky ureido congener. Certain congeners were up to 145-fold more active at A_1 receptors than at A_2 receptors. Various derivatives of the congeners should be useful as receptor probes and for radioiodination, avidin binding, and preparation of affinity columns.

Alkylxanthines, with theophylline (**1**) as the prototype, represent the major class of antagonists for adenosine receptors.[1] Although theophylline and caffeine are relatively weak adenosine antagonists, with affinity constants in the 10–50 μM range, they undoubtedly owe many of their pharmacological effects to blockade of adenosine-mediated functions. Two classes of adenosine receptors have been characterized.[1] The A_1-adenosine receptor is inhibitory to adenylate cyclase and appears involved in antilipolytic, cardiac depressant, and central depressant effects of adenosine analogues. The A_2-adenosine receptor is stimulatory to adenylate cyclase and appears involved in hypotensive, vasodilatory, antithrombotic, and endocrine effects of adenosine analogues. Xanthines, by blockade of adenosine binding at such receptors, would have the opposite effects. Some xanthines, such as 3-isobutyl-1-methylxanthine, not only block adenosine receptors but also have potent inhibitory effects on phosphodiesterases.[2a] The presence of an 8-phenyl group greatly enhances the potency of theophylline as an adenosine antagonist.[2a,b] Even more potent antagonists result from the replacement of the 1,3-methyl groups of 8-phenyltheophylline with *n*-propyl groups and by situating uncharged electron-donating para substituents on the 8-phenyl ring. Affinity constants (K_i) of less than 1 nM at A_1-adenosine receptors have been attained.[3,22] In addition to high potency, some selectivity toward the A_1 subclass of adenosine receptors has been obtained with 1,3-dipropyl-8-phenylxanthines.[4]

1,3-Dipropyl-8-(*p*-hydroxyphenyl)xanthine (**2b**) was chosen as a suitable lead compound in an effort to develop potent and selective functionalized congeners as antagonists for adenosine receptors. An N^6-phenyladenosine was chosen as a suitable lead compound for a parallel effort to develop potent and selective agonists for A_1-adenosine receptors.[5] In the design of active, covalent conjugates of drugs, the goals of the functionalized congener approach are several, including increasing the potency, prolonging the duration of action, and/or changing the specificity. This approach has been applied to isoproterenol and has lead to analogues with activity at β-adrenergic receptors as much as 4 orders of magnitude greater than that of isoproterenol.[6-8] Nontherapeutic applications of active, functionalized drugs include receptor probes,[5,9,10] immobilized ligands for affinity chromatography,[11] and radiolabeled analogues.

We report here the synthesis of a series of highly potent analogues of theophylline and 1,3-dipropylxanthine, some of which contain groups designed for radiolabeling through the introduction of radioisotopes of iodine. The functionalized congeners are also suitable for the preparation of affinity columns. A biotinylated conjugate is intended for use as a molecular probe through linkage to avidin.[12a]

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