70977-53-6; **3y**, 70977-43-4; **3z**, 70977-45-6; **4** (R^1 = CH_3CO, R^2 = Cl), 1760-85-6; **4** (R^1 = CH_3CO, R^2 = Br), 1760-84-5; [4] (R^1 = CH_3CO, R^2 = CH_3O), 55008-13-4; **4** (R^1 = C_2H_5CO, R^2 = CH_3), 1760-86-7; **4** (R^1 = R^2 = CH_3O), 61637-60-3; **4** (R^1 = CH_3O, R^2 = NO_2), 20718-78-9; **4** (R^1 = CH_3O, R^2 = $NHCOCH_3$), 100245-39-4; **4** (R^1 = H, R^2 = Br), 89-55-4; **4** (R^1 = H, R^2 = CN), 10435-57-1; **4** (R^1 = H, R^2 = CH_3S), 32318-42-6; **4** (R^1 = H, R^2 = C_6H_5), 323-87-5; **4** (R^1 = H, R^2 = CH_3CO), 13110-96-8; **4** (R^1 = H, R^2 = $NHCOCH_3$), 51-59-2; **4** (R^1 = CH_3CO, R^2 = CH_3), 1760-83-4; **4** (R^1 = CH_3CO, R^2 = CH_3) (acid chloride), 70986-26-4; **4** (R^1 = CH_3O, R^2 = CH_3), 4386-42-9; **4** (R^1 = H, R^2 = F), 345-16-4; **4** (R^1 = H, R^2 = *t*–C_4H_9), 16094-31-8; **4** (R^1 = H, R^2 = CH_3O), 2612-02-4; **4** (R^1 = H, R^2 = NO^2), 96-97-9; **4** (R^1 = H, R^2 = NO_2) (acid chloride), 3223-20-9; **4** (R^1 = CH_3O, R^2 = Br), 35090-76-7; **4a**, 67127-78-0; **4b**, 67127-75-7; **4c**, 67127-80-4; **4d**, 100245-30-5; **4e**, 67127-77-9; **4f**, 67127-79-1; **4g**, 100245-36-1; **4h**, 100245-37-2; **4i**, 67127-81-5; **4j**, 100245-34-9; **4k**, 100245-35-0; **4l**, 100297-40-3; **4m**, 3507-08-2; **4n**, 100245-33-8; **5** (R^1 = H, R^2 = CH_3), 67127-92-8; **5** (R^1 = R^2 = H), 14389-86-7; **5** (R^1 = H, R^2 = Cl), 52803-75-5; **5** (R^1 = H, R^2 = CF_3), 53985-54-9; **5** (R^1 = H, R^2 = $PhCH_2O$), 67127-91-7; **6a**, 100245-38-3; **6b**, 67127-71-3; **6c**, 67127-68-8; **6d**, 67127-74-6; **6e**, 67127-70-2; **7a**, 100245-22-5; **7aa**, 70977-95-6; **7ab**, 70978-13-1; **7ac**, 70978-11-9; **7ad**, 70978-12-0; **7ae**, 100245-28-1; **7af**, 100245-29-2; **7b**, 70977-74-1; **7c**, 70977-89-8; **7d**, 70978-15-3; **7e**, 70977-76-3; **7f**, 100245-23-6; **7g**, 100245-24-7; **7h**, 70977-96-7; **7i**, 100245-25-8; **7j**, 70977-97-8; **7k**, 70977-98-9; **7l**, 70977-93-4; **7m**, 70977-90-1; **7n**, 70978-01-7; **7o**, 100245-26-9; **7p**, 70977-91-2; **7q**, 70977-99-0; **7r**, 70978-02-8; **7s**, 70978-17-5; **7t**, 70978-03-9; **7u**, 70977-73-0; **7v**, 100245-27-0; **7w**, 70978-14-2; **7x**, 70977-92-3; **7y**, 70977-94-5; **7z**, 70978-00-6; **8** (R^1 = CH_3CO, R^2 = C_2H_5, Ar = C_6H_5), 70978-21-1; **8** (R^1 = CH_3CO, R^2 = C_{H5}, Ar = *P*–$CH_3OC_6H_4$), 70978-04-0; **9a**, 70978-25-5; **9b**, 100245-21-4; **9c**, 70978-26-6; **9d**, 70978-27-7; **9e**, 70978-18-6; **9f**, 70978-28-8; **9g**, 70978-30-2; **9h**, 70978-29-9; **10** (R^1 = CH_3CO, R^2 = Cl), 21312-85-6; **10** (R^1 = CH_3CO, R^2 = CH_3O), 55008-15-6; **10** (R^1 = CH_3oCO, R^2 = $NHCOCH_3$), 70978-63-1; **10** (R^1 = CH_oCO, R^2 = H), 70977-72-9; **10** (r^1 = CH_3CO, R^2 = *t*–C_{H9}), 100245-20-3; **10** (R^1 = C_2H_5CO, R^2 = C_2H_5), 70978-24-4; **10** (R^1 = C_2H_5CO, R^2 = CH_3), 70978-23-3; **10** (R^1 = CH_3CO, R^2 = CH_3SO_2), 70977-88-7; **10a**, 70977-71-8; **10b**, 70977-78-5; **10c**, 70978-22-2; **10d**, 70977-84-3; **10e**, 70977-85-4; **10f**, 70977-81-0; **10g**, 70977-79-6; **10h**, 100245-11-2; **10i**, 70977-86-5; **10j**, 24962-75-2; **10k**, 70978-64-2; **10l**, 70977-80-9; **10m**, 70977-82-1; **10n**, 70977-87-6; **10o**, 70977-83-2; **10p**, 70978-09-5; **10q**, 70978-07-3; **10r**, 70978-08-4; **10s**, 100245-12-3; **10t**, 100245-13-4; **11**, 70978-65-3; **12**, 55408-10-1; **13a**, 63005-72-1; **13a** (benzyl ester), 100245-14-5; **13a** (acid chloride), 64470-37-7; **13b**, 70977-77-4; **13b** (benzyl ester), 100245-16-7; **13b** (acid chloride), 66492-66-8; **14a**, 71002-71-6; **14b**, 70978-38-0; **14c**, 100245-06-5; **14d**, 70978-39-1; **14e**, 70978-59-5; **14f**, 70978-46-0; **14g**, 100245-07-6; **14h**, 70978-47-1; **14i**, 70978-55-1; **14j**, 70978-60-8; **14k**, 42247-91-6; **14l**, 70978-45-9; **14m**, 70978-42-6; **14n**, 70978-49-3; **14o**, 70978-50-6; **14p**, 70978-48-2; **14q**, 70978-44-8; **14r**, 67191-44-0; **14s**, 70978-43-7; **14t**, 100245-08-7; **14u**, 100245-09-8; **14**, 100245-10-1; **14w**, 70978-51-7; **14x**, 70978-52-8; **14y**, 70978-53-9; *p*-$CH_3OC_6H_4COCl$, 824-94-2; 2-acetyl-4-ethylphenol, 24539-92-2; 2-acetyl-4-propylphenol, 1990-24-5; 2-acetyl-4-*tert*-butylphenol, 57373-81-6; 2-acetyl-4-fluorophenol, 394-32-1; 2-acetyl-4-cyanophenol, 35794-84-4; 2-acetyl-4-(methylsulfonyl)-phenol, 20951-24-0; 2,4-diacetylphenol, 30186-16-4; 2-(1-oxopropyl)-4-ethylphenol, 63909-10-4; 2-(1-oxopropyl)-4-cyanophenol, 70978-58-4; 2-methoxy-4-ethylphenol, 2785-89-9; 4-(dimethylaminosulfonyl)phenol, 15020-57-2; 2-(1-oxobutyl)-4-methylphenol, 24323-47-5; 2-(1-oxoisobutyl)-4-methylphenol, 64207-03-0; 2-(cyclopropylcarbonyl)-4-methylphenol, 70978-56-2; 2-(benzylcarbonyl)-4-methylphenol, 24258-63-7; 2-(trifluoroacetyl)-4-methylphenol, 70978-57-3; 2-(dimethylaminocarbonyl)-4-methylphenol, 100245-03-2; methyl 2-hydroxy-5-methylbenzoate, 22717-57-3; ethyl 2-hydroxy-5-methylbenzoate, 34265-58-2; 2-(5-tetrazolyl)-4-methylphenol, 100245-04-3; 2-(4-methoxyphenylmethylaminocarbonyl)-4-methylphenol, 100245-05-4; 2-methoxy-6-nitrophenol, 15969-08-1; 2-methoxy-4-methyl-6-nitrophenol, 53411-80-6; 2-methoxy-4-bromo-6-nitrophenol, 70978-61-9; 2-methoxyphenol, 90-05-1; 4-methyl-2-methoxyphenol, 93-51-6; 4-bromo-2-methoxyphenol, 7368-78-7; 4-methyl-2-methoxy-6-nitrophenol, 66108-30-3; 4-bromo-2-methoxy-6-nitrophenol, 70978-54-0; 2-acetyl-4,6-dinitrophenol, 69027-37-8; ethyl cyanoformate, 623-49-4; potassium 2-benzyltetrazole-5-carboxylate, 70978-32-4; benzyl 2-benzyltetrazole-5-carboxylate, 100245-15-6; 2-benzyltetrazole-5-carbonylchloride, 100245-18-9; potassium 2-(*p*-methoxybenzyl)tetrazole-5-carboxylate, 70978-33-5; benzyl 2-(*p*-methoxybenzyl)tetrazole-5-carboxylate, 100245-17-8; 2-(*p*-methoxybenzyl)tetrazole-5-carbonyl chloride, 100245-19-0; tetracyanoethylene oxide, 3189-43-3; carbonyldicyanide, 1115-12-4; 3-methoxy-2-(benzyloxy)benzenamine, 70978-05-1; 5-methyl-3-methoxy-2-(benzyloxy)benzenamine, 70978-19-7; 5-ethyl-3-methoxy-2-(benzyloxy)benzenamine, 70978-06-2; 5-bromo-3-methoxy-2-(benzyloxy)benzenamine, 70978-31-3; 2-(benzyloxy)-benzenamine, 20012-63-9; 2-hydroxy-3-propenylacetophenone, 67127-96-2; 3-formyl-2-hydroxyacetophenone, 55108-29-7; 3-allyl-2-hydroxyacetophenone, 58621-39-9; 2-acetoxy-5-ethylbenzoic acid, 35421-90-0; 2-acetoxy-5-fluorobenzoic acid, 448-40-8; (1-oxopropyl)-5-ethylbenzoic acid, 67127-93-9; 5-propylsalicyclic acid, 28488-44-0; 5-isobutylsaslicyclic acid, 100245-31-6; 5-(*tert*-butyl)salicyclic acid, 16094-31-8; 5-ethyl-2,3-dihydroxybenzoic acid, 100245-32-7; 2-hydroxy-3-methoxybenzoic acid, 877-22-5; methyl 5-methylsalicylate, 22717-57-3; 5-tetrazolamine, 4418-61-5; 3-acetyl-2-hydroxy-5-(chlorosulfonyl)-*n*-(1*h*-tetrazol-5yl)benzamide, 100245-52-1; 5-(chlorosulfonyl)-2-hydroxy-*n*-(1*h*-tatrazol-5-yl)-benzamide, 100245-53-2; *n*-(cyanocarbonyl)-2-(benzyloxy)-benzenamine, 100297-41-4; *n*-(5-tetrazoylcarbonyl)-2-(benzyloxy)benzenamine, 100245-57-6.

Quantitative Evaluation of the β_2-Adrenoceptor Intrinsic Activity of *N-tert*-Butylphenylethanolamines†

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The extent of stimulation of the enzyme adenylate cyclase, and the concomitant production of cAMP, by a number of β-adrenoceptor agonists, all belonging to the class of the *N-tert*-butylphenylethanolamines, has been determined. The results have been used as direct measures for intrinsic sympathomimetic activity (ISA) and were correlated with various physicochemical parameters of the compounds. Significant correlations were established by means of the method of multiple regression analysis, and it was demonstrated that electronic effects only govern ISA. The use of ^{13}C NMR chemical shifts of the aromatic C atoms proved to be a valuable tool in this analysis.

In 1954, Ariëns[1] introduced the concept of intrinsic activity, as a necessary completion to the receptor-occupation theory, originally proposed by Clark.[2] Further refinements were made by Furchgott,[3] Nickerson,[4] and Stephenson,[5] and now it is generally believed that intrinsic activity does

† Dedicated to Jan van Dijk (Duphar, Weesp, The Netherlands) on the occasion of his retirement.

(1) Ariëns, E. J. *Arch. Int. Pharmacodyn.* **1954**, *99*, 32.
(2) Clark, A. J. *J. Physiol. (London)* **1926**, *61*, 547.
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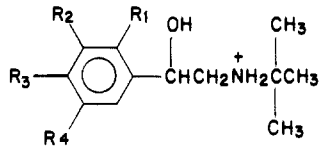
Table I. β_2-Adrenoceptor Affinities and Intrinsic Activities of Substituted *N-tert*-Butylphenylethanolamines



no.	compd	R_1	R_2	R_3	R_4	"true" $-\log K_D$[a]	% cations present at pH 8.0[b]	app K_D at pH 8.0,[c] μM	[ligand] for 95–100% receptor occupancy[d]	cAMP production[e]
1	(±)-Th 1206	H	OH	OH	H	6.55	84	0.67	3.10^{-5}	83 ± 4
2	(±)-terbutaline	H	OH	H	OH	5.73	83	4.48	1.10^{-4}	50 ± 5
3	(±)-*N-tert*-butylnorsynephrine	H	H	OH	H	6.20	93	1.35	1.10^{-4}	97 ± 5
4	(±)-Du 28663	H	NH_2	OH	H	5.99	94	2.17	1.10^{-4}	83 ± 6
5	(±)-SKF 56301	H	$NHCH_3$	OH	H	6.58	94	0.56	3.10^{-5}	115 ± 3
6	(±)-salbutamol	H	CH_2OH	OH	H	6.58	92	0.57	3.10^{-5}	122 ± 9
7	(±)-AH 3474	H	$CONH_2$	OH	H	7.03	18	1.03	3.10^{-5}	24 ± 6
8	(±)-clenbuterol	H	Cl	NH_2	Cl	7.74	98	0.037	1.10^{-6}	64 ± 7
9	(±)-C 78	Cl	H	H	H	7.18	98	0.13	1.10^{-5}	61 ± 9
10	(±)-VUF 8303	H	Cl	OH	Cl	7.41	1.2	6.47	3.10^{-4}	47 ± 5
11	(±)-*N-tert*-butylphenylethanolamine	H	H	H	H	5.84	98	2.94	1.10^{-4}	43 ± 5

[a] Values taken from ref 14. [b] As derived from the macroscopic ionization constants. [c] Calculated as described in Results. [d] Concentrations in M. [e] Values are in pmol (mg of protein)$^{-1}$ min^{-1} ± SEM (n = 5–6).

not need to be linearly proportional to the percentage of receptors occupied. In this concept, full agonists are defined as substances that produce maximal effects in functional studies (e.g., with isolated organ preparations) without necessarily occupying 100% of the receptors. This phenomenon is known as receptor reserve, which inherently obscures the real affinity of agonists for their receptors. Another problem in quantitatively defining drug effects is the impossibility of discriminating between full agonists with respect to intrinsic activity, all having an intrinsic activity of 1.0.

The finding that cAMP should be considered as the "second messenger" following the hormone–β-adrenoceptor interaction,[6] the development of sensitive assays for the determination of adenylate cyclase activity,[7] and the introduction of the radioligand binding technique[8] have provided additional tools to overcome the problems mentioned above.

Firstly, it was shown that K_D values, as derived from radioligand binding studies, are direct measures for β-adrenoceptor affinity.[9] Secondly, K_D values of agonists proved to be equal to K_A values, the drug constant for activation of the enzyme adenylate cyclase,[10] which strongly suggests the absence of any receptor reserve for the cAMP production, at least in cell membrane preparations. Consequently, the extent of adenylate cyclase activation is a direct and unambiguous measure for the intrinsic activity of β-adrenoceptor agonists (ISA: intrinsic sympathomimetic activity).

This feature offers a perspective for an analysis relating intrinsic activities with physicochemical characteristics of β-adrenoceptor agonists, and, indeed, some attempts have already been made.

Dunn and co-workers[11] have been able to correctly classify agonists and antagonists by the SIMCA method of pattern recognition, based on data from Mukherjee et al.[12] obtained on the frog erythrocyte β-adrenoceptor. Intrinsic activities in the latter study, however, are probably underestimated, due to the absence of GTP, a necessary cofactor in the coupling between β-adrenoceptor and adenylate cyclase, in the assay. Bilezikian et al.[10] have performed similar studies on the turkey erythrocyte β-adrenoceptor, now in the presence of GppNHp, a stable analogue of GTP. These authors concluded that a catechol moiety, as present in the endogenous ligands noradrenaline and adrenaline, is a prerequisite for high intrinsic activity. Futhermore, a low correlation was noticed between ISA and K_D values of the derivatives examined. Šolmajer et al.[13] have used Bilezikian's data in order to establish a correlation between ISA and molecular electrostatic potentials, which will be referred to in the Discussion.

Recently, we have quantitatively evaluated the factors that control β_2-adrenoceptor affinity using a membrane preparation of a bovine skeletal muscle.[14] From the performed regression analyses, relating K_D values of three classes of β-adrenoceptor ligands with various physicochemical parameters, it was concluded that lipophilicity and steric factors are of prime importance in the definition of the ligand–β_2-adrenoceptor interaction with respect to affinity. Interested in intrinsic activity as well, we now present a quantitative evaluation of this biological parameter in the same membrane preparation for the class of *N-tert*-butylphenylethanolamines, containing well-known antiasthma drugs, like salbutamol and terbutaline.

Results

In Table I the structures of the derivatives considered, all belonging to the class of the *N-tert*-butylphenylethanolamines, are given, together with biological data,

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(7) Salomon, Y.; Londos, C.; Rodbell, M. *Anal. Biochem.* **1974**, *58*, 541. Also: Torey, K. C.; Oldham, K. G.; Whelan, J. A. M. *Clin. Chim. Acta* **1974**, *56*, 221.
(8) See for instance: Aurbach, G. D.; Fedak, S. A.; Woodard, C. J.; Palmer, J. S.; Hauser, D.; Troxler, F. *Science (N.Y.)* **1974**, *186*, 1223. Also: Lefkowitz, R. J.; Mukherjee, C.; Coverstone, M.; Caron, M. G. *Biochem. Biophys. Res. Commun.* **1974**, *60*, 703.
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(10) Bilezikian, J. P.; Dornfeld, A. M.; Gammon, D. E. *Biochem. Pharmacol.* **1978**, *27*, 1445.
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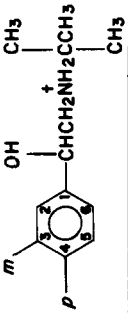
(12) Mukherjee, C.; Caron, M. G.; Mullikin, D.; Lefkowitz, R. J. *Mol. Pharmacol.* **1976**, *12*, 16.
(13) Šolmajer, T.; Hodošček, M.; Hadži, D.; Lukovits, I.; *Quant. Struct.-Act. Relat.* **1984**, *3*, 51.
(14) IJzerman, A. P.; Auë, G. H. J.; Bultsma, T.; Linschoten, M. R.; Timmerman, H. *J. Med. Chem.* **1985**, *28*, 1328.

representing their affinity for and their intrinsic activity on β_2-adrenoceptors. The "true" $-\log K_D$ values of compounds 1–10, given in Table I, are taken from one of our previous studies.[14] In that report it was shown that apparent K_D values, determined at pH 7.5, needed to be corrected in two ways in order to obtain "true" $-\log K_D$ values as a reflection of real β-adrenoceptor affinity. In the first place, apparent $-\log K_D$ values were adjusted for the amounts of cations present at pH 7.5, as this ionic species was shown to govern β_2-adrenoceptor affinity.[15] Secondly, as only the (−)-isomers of the racemates in Table I are thought to be active on β-adrenoceptors,[16] a further correction was performed. From these "true" $-\log K_D$ values the concentrations of ligands necessary for a maximal cAMP production in this membrane preparation, as a direct measure for intrinsic activity, are easily calculated. As the stimulation of the enzyme adenylate cyclase proved to be best pronounced at pH 8.0 in our preparation, the percentages cations of the various ligands at pH 8.0 were calculated according to their macroscopic ionization constants, determined as in ref 17. From "true" $-\log K_D$ values and the amounts of cations present at pH 8.0, the apparent K_D values of compounds 1–10 adjusted for the presence of racemates only were calculated, which, in turn, were used to determine the concentrations of β-adrenoceptor agonists necessary for (nearly) maximal receptor occupancy (see also Experimental Section). These concentrations of agonists were included in the cAMP assay; the resulting cAMP production is shown in the last column of Table I (cAMP production of 3×10^{-5} M (±)-isoprenaline—not belonging to the investigated class—was 90 ± 5 pmol (mg of protein)$^{-1}$ min^{-1}.

Two assumptions are implicit in this reasoning. Firstly, the "true" affinity of β-adrenoceptor ligands does not vary to considerable extent between pH 7.5 and 8.0. In fact, this was shown to be the case, as the affinity of the radioligand [^{3}H]dihydroalprenolol, a ligand with only minimal variation in cation concentration at these two pH values, changes only slightly.[15] Secondly, the equilibrium constants for activation (K_A) and binding/dissociation (K_D) should be virtually identical. This has proven to be true, as has been mentioned, in a number of studies with several membrane preparations and a large number of β-adrenoceptor ligands.[10,12] The results given in Table I were checked by using three times higher concentrations, which did not yield higher values for cAMP production (results not shown).

In Table II we have summarized the biological and physicochemical parameters, which were explored in a multiple regression analysis (for explanation of symbols see Experimental Section and legend to Table II). Although the intrinsic activities (ISA) are all relative to salbutamol, and thus given as fractions of maximal intrinsic activity, they represent a certain amount of cAMP production, expressed in pmol (mg of protein)$^{-1}$ min^{-1}. Therefore, the application of this biological parameter as such, seems to be questionable in putative linear free-energy relationships. Consequently, we have considered log (1/ISA) as a descriptor in biological activity as well, as suggested by De Jonge et al.[18] However, since both pa-

Table II. Intrinsic Sympathicomimetic Activities and Physicochemical Parameters of Substitued *N-tert*-Butylphenylethanolamines



no.	ISA[a]	log (1/ISA)	σ_m	F_m	R_m	σ_p	Sb_m	Sb_p	$\Delta C_1'^b$	ΔC_2	ΔC_3	ΔC_4	ΔC_5	ΔC_6	$(\Delta C_1 + \Delta C_5)$	log P_{calcd}	ISA (acc to eq 10)	log (1/ISA) (acc to eq 11)
1	0.68	0.17	0.12	0.29	−0.64	−0.37	1.0	1.0	3.7	−14.7	15.5	15.5	−12.3	−10.1	−8.6	1.030	0.78	0.13
2	0.41	0.39	0.12	0.29	−0.64	0.00	1.0	0.0	14.5	−23.6	28.6	−26.0	28.6	−23.6	43.1	0.741	0.38	0.39
3	0.80	0.10	0.00	0.00	0.00	−0.37	0.0	1.0	3.0	−1.0	−12.9	27.1	−12.9	−1.0	−9.9	1.526	0.75	0.14
4	0.68	0.17	−0.16	0.02	−0.68	−0.37	1.0	1.0	6.7	−12.2	6.6	13.3	−11.3	−9.3	−4.6	0.502	0.76	0.13
5	0.95	0.02	−0.30	−0.11	−0.74	−0.37	2.0	1.0	0.1	−11.1	0.1	20.9	−11.1	−7.8	−11.0	1.098	0.83	0.08
6	1.00	0.00	0.00	0.00	0.00	−0.37	2.0	1.0	3.0	−1.6	−1.7	25.4	−12.6	−1.6	−9.6	0.682	0.77	0.12
7	0.20	0.70	0.28	0.24	0.14	−0.37	3.0	1.0	2.5	−2.1	−13.2	29.4	−11.0	3.4	−8.5	1.103	0.21	0.66
8	0.53	0.28	0.37	0.41	−0.15	−0.66	1.2	1.0	3.9	−2.8	−7.1	9.8	−7.1	−2.8	−3.2	3.060	0.66	0.21
9	0.50	0.30	0.00	0.00	0.00	0.00	0.0	0.0	13.2	5.0	0.2	−0.2	−2.0	−0.4	11.2	2.764	0.57	0.24
10	0.39	0.41	0.37	0.41	−0.15	−0.37	1.2	1.0	5.5	−1.2	−5.9	19.6	−5.9	−1.2	−0.4	3.010	0.53	0.32
11	0.35	0.46	0.00	0.00	0.00	0.00	0.0	0.0	13.0	−1.4	0.0	−1.2	0.0	−1.4	13.0	2.022	0.23	0.58

[a] ISA: intrinsic sympathicomimetic activity, relative to salbutamol (= 1.0). [b] $\Delta C_1 = \delta(C_{1\,compound}) - \delta(C_{1\,benzene})$ (in ppm) = $\delta(C_{1\,compound}) - 128.5$, as derived from ^{13}C NMR data.

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rameters are not related to free energies, we will further avoid the term linear free-energy relationships. The regression equations to be shown should be seen as mere mathematical descriptions.

In a first attempt, we have tried to correlate ISA or log (1/ISA) with log P_{calcd} (partition coefficient octanol/water) of the aromatic moieties of the compounds 1–10, on the assumption that the influence of the (identical) side chain on ISA is similar for all derivatives. However, correlation proved to be very poor for log P_{calcd} alone (r = –0.3624) or in combination with various other physicochemical parameters (regression equations not shown).

Similar conclusions were drawn with respect to Sb, an easily calculated steric parameter for any conceivable substituent.[27] Neither for substituents meta nor for substituents para to the aliphatic side chain (designated as m and p in Table II) could a steric influence on intrinsic activity be demonstrated (ISA vs. Sb_m, r = –0.1140, ISA vs. Sb_p, r = 0.3277). In contrast, a correlation explaining 50% of the observed variance was established with electronic parameters, viz. Hammett's σ. For substituents R_2 and R_3 in Table I we have taken σ_m and σ_p, respectively.[29] The following equations were obtained (results for ISA only):

$$\text{ISA} = -0.83(\pm0.67)\sigma_m + 0.68(\pm0.14) \quad (1)$$

$$n = 10,\ r = 0.7092,\ s = 0.1912,\ F = 8.094$$

$$\text{ISA} = -0.95(\pm0.61)\sigma_m - 0.56(\pm0.71)\sigma_p + 0.51(\pm0.26) \quad (2)$$

$$n = 10,\ r = 0.7917,\ s = 0.1771,\ F = 7.052$$

It should be mentioned that the introduction of σ_p is debatable, as only three substituents (H, OH, and NH_2) occur in this position (cf. the 95% confidence interval of σ_p).

From the intercept in both equations (0.68 and 0.51, respectively) it appeared that the unsubstituted *N-tert*-butylphenylethanolamine (11) would have intrinsic activity on β_2-adrenoceptors as well. Thus, we have synthesized this compound and tested it for affinity and intrinsic activity. In a concentration of 1×10^{-4} M, sufficient to occupy more than 95% of the β_2-adrenoceptors, this derivative produced a (maximal) cAMP level of 43 ± 5 pmol (mg of protein)$^{-1}$ min^{-1}, being 0.35 times the intrinsic activity of salbutamol.

With compound 11 included, the eq 3–6 were derived. Stimulated by these results, we have further attempted to elucidate the electronic requirements for intrinsic activity.

$$\text{ISA} = -0.77(\pm0.70)\sigma_m + 0.65(\pm0.16) \quad (3)$$

$$n = 11,\ r = 0.6334,\ s = 0.2082,\ F = 6.030$$

$$\log(1/\text{ISA}) = 0.61(\pm0.61)\sigma_m + 0.23(\pm0.14) \quad (4)$$

$$n = 11,\ r = 0.6080,\ s = 0.1756,\ F = 5.279$$

$$\text{ISA} = -0.96(\pm0.60)\sigma_m - 0.67(\pm0.60)\sigma_p + 0.46(\pm0.21) \quad (5)$$

$$n = 11,\ r = 0.7978,\ s = 0.1721,\ F = 8.227$$

$$\log(1/\text{ISA}) = 0.75(\pm0.55)\sigma_m + 0.49(\pm0.55)\sigma_p + 0.36(\pm0.18) \quad (6)$$

$$n = 11,\ r = 0.7354,\ s = 0.1590,\ F = 5.678$$

Swain and Lupton[19] defined polar (F) and resonance (R) constants in order to separate the inductive from the resonance component in the electronic effect of substituents. The intercorrelation between F_m and σ_m in our dataset is far higher (r = 0.9042) than between R_m and σ_m (r = 0.4413); as a consequence, the regression equation with F_m instead of σ_m equally describes intrinsic activity for all 11 compounds (eq 7).

$$\text{ISA} = -0.74(\pm0.86)F_m + 0.69(\pm0.20) \quad (7)$$

$$n = 11,\ r = 0.5482,\ s = 0.2251,\ F = 3.867$$

The inclusion of R_m improves the quality of eq 7 only marginally (r = 0.5809). Furthermore, we have incorporated ^{13}C NMR chemical shifts in our analyses. Again, on the assumption, that the influence of the aliphatic side chain on intrinsic activity is identical for all compounds, we have limited ourselves to the chemical shifts of the C atoms of the aromatic moiety. As has been mentioned, the cationic species governs β_2-adrenoceptor affinity, and therefore all ^{13}C NMR spectra were recorded with the solutes in D_2O, acidified with HCl (pD ≈ 4). Under these conditions only cations are present. From the chemical shifts a value of 128.5 ppm was subtracted, being the chemical shift of the C atoms in benzene. In this way, substituent effects on all C atoms of the aromatic moiety were quantified and are given as ΔC values in Table II. In eq 8–11 only ΔC values were introduced, as it was felt improper to combine these values with other (electronic) parameters. The sum of the values for ΔC_1 and ΔC_5,

$$\text{ISA} = 0.068(\pm0.061)\Delta C_2 + 0.025(\pm0.017)\Delta C_4 - 0.111(\pm0.083)\Delta C_6 + 0.133(\pm0.315) \quad (8)$$

$$n = 11,\ r = 0.7717,\ s = 0.1941,\ F = 4.877$$

$$\log(1/\text{ISA}) = -0.064(\pm0.050)\Delta C_2 - 0.020(\pm0.012)\Delta C_4 + 0.102(\pm0.069)\Delta C_6 + 0.646(\pm0.255) \quad (9)$$

$$n = 11,\ r = 0.7768,\ s = 0.1579,\ F = 5.022$$

$$\text{ISA} = 0.062(\pm0.047)\Delta C_2 - 0.023(\pm0.012)[\Delta C_1 + \Delta C_5] - 0.099(\pm0.064)\Delta C_6 + 0.484(\pm0.137) \quad (10)$$

$$n = 11,\ r = 0.8464,\ s = 0.1625,\ F = 7.951$$

$$\log(1/\text{ISA}) = -0.062(\pm0.028)\Delta C_2 + 0.020(\pm0.007) \times [\Delta C_1 + \Delta C_5] + 0.097(\pm0.038)\Delta C_6 + 0.369(\pm0.085) \quad (11)$$

$$n = 11,\ r = 0.9175,\ s = 0.0997,\ F = 16.103$$

termed $[\Delta C_1 + \Delta C_5]$, was used, since the regression coefficients for ΔC_1 and ΔC_5 proved to be similar in earlier equations (results not shown).

Of course, the intercorrelation between ΔC_4 and $[\Delta C_1 + \Delta C_5]$ is considerable (r = –0.9516). On the other hand, the intercorrelations between σ_m and the various ΔC values are very low, σ_m vs. ΔC_6 (r = 0.2135) being the highest. In

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Table II, all estimated values for ISA and log 1/ISA are according to the eq 10 and 11, respectively.

Discussion

The following conclusions are to be drawn from the regression equations. Lipophilicity is not involved in β_2-adrenoceptor intrinsic activity nor are steric parameters. Previously, we have reported on the characteristics of the ligand–β_2-adrenoceptor interaction with respect to affinity.[14] In that study we have derived several regression equations, relating K_D values with various physicochemical parameters. For the class of compounds, examined in the present study, we have found eq 12 (compounds 1–10).

$$-\log K_D = 0.63(\pm 0.17) \log P_{calcd} + 0.27(\pm 0.14)[Sb_m + Sb_p] + 5.17(\pm 0.47) \quad (12)$$

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

This equation clearly demonstrates the importance of both lipophilic and steric characteristics within the class of the *N-tert*-butylphenylethanolamines with regard to β_2-adrenoceptor affinity. Thus, a major statement should be, that affinity and intrinsic activity are two phenomena that are completely separated and unrelated, although both are of prime importance in the description of the agonist–β_2-adrenoceptor interaction.

In contrast to lipophilicity and steric parameters, electronic effects appear to be primarily responsible for the occurrence of intrinsic activity (eq 1–6). Of σ_m and σ_p, the former parameter seems to be most relevant, and from eq 7 it is suggested that the influence of σ_m is best explained by the assumption of inductive (polar) effects (F) rather than resonance (R). The hypothesis, as derived from the eq 1 and 2, that the unsubstituted *N-tert*-butylphenylethanolamine, too, should have some intrinsic activity was confirmed. To our knowledge, this finding has not been reported thus far. In an earlier study, this compound was shown to possess α-amylase inhibitory activity in mice salivary glands.[20] The corresponding unsubstituted *N*-isopropylphenoxypropanolamine exhibits strong intrinsic activity (50% relative to isoprenaline), as was demonstrated by ICI investigators in an in vivo rat model.[21] Obviously, the idea that polar substituents, such as a phenolic OH group, are necessary for intrinsic activity needs to be shaded somewhat.

The electronic aspects of intrinsic activity were further investigated by ^{13}C NMR of the *N-tert*-butylphenylethanolamines. It was thought that the chemical shifts of the C atoms in the phenyl nucleus would provide insight in the charge distribution (π-electron density) on this aromatic moiety, as the paramagnetic term (defining the excitation of π-electrons) prevails in ^{13}C NMR.[22] Most probably, other factors determining chemical shift data, like steric and direct magnetic effects, are constant within this structurally congeneric class of derivatives. The implicit influence of all aromatic substituents on the chemical shifts of the phenyl C atoms is a further advantage, since, as a matter of fact, R_1 and R_4 (see Table I) are also taken into account.

Introduction of ΔC values, as in the eq 8–11, leads to a further improvement in the quality of the regression equations. In all these equations three independent variables were used, which at first glance seems abundant for the examination of 11 compounds only.

All ΔC values, however, have the same physical origin, and in our opinion, the concomitant presence of these parameters is thus justified.

In eq 8 and 9, ΔC_2, ΔC_4, and ΔC_6 are combined, and these values probably reflect the electronic influence of the substituent meta to the aliphatic side chain, for C_2, C_4, and C_6 are the positions, ortho and para to this substituent, that are likely to be influenced most by substitution at R_2. The better quality of eq 10 and 11 in comparison to eq 8 and 9 suggests that the other substituents on the aromatic nucleus are relevant as well. Nevertheless, it should be recognized, of course, that the spectroscopic measurements are performed under conditions not corresponding to the biological work, although the presence in D_2O of the active cationic species only, has been warranted.

As has been mentioned, other investigators made some attempts to describe intrinsic activity as a function of other characteristics of β-adrenoceptor ligands. In an important study on the β-adrenoceptor, present on membranes of the turkey erythrocyte, Bilezikian et al.[10] demonstrated low but significant correlations between the intrinsic activity of a large number of agonists and their K_D (or K_A) values. Correlating the ISA values from Table II with the "true" $-\log K_D$ values of Table I, we did not observe any connection between the two biological parameters:

$$\mathrm{ISA} = 0.025(\pm 0.088)[-\log K_D] + 0.43(\pm 0.57) \quad (13)$$

$$n = 11,\ r = 0.2102,\ s = 0.2631,\ F = 0.416$$

an indication, again, that both phenomena are completely independent.

On the basis of the results of Bilezikian et al., Šolmajer et al.[13] found a relationship between ISA and V_A, in which V_A is defined as the calculated value of the molecular electrostatic potentials (MEP) in a certain point A, which, in our data set, would be located somewhere between the two phenolic OH groups of Th 1206 (compound 1):

$$\mathrm{ISA} = 0.502(\pm 0.135) V_A + 0.079(\pm 0.009) \quad (14)$$

$$n = 52,\ r = 0.60,\ s = 0.25,\ F = 13.7$$

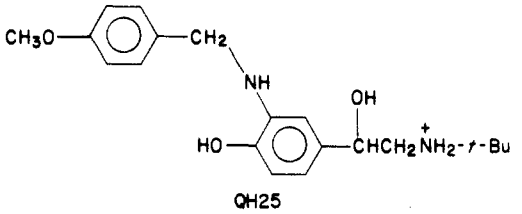
The authors conclude that electrostatic properties of the aromatic region of the ligands are related to intrinsic activity, a concept that was earlier suggested by Petrongolo et al.[23]

Although the choice of point A is rather arbitrary, results seem to be compatible with our findings in a more general way: electronic effects, important in our analysis, will influence electrostatic potentials and hence V_A.

How should these results be interpreted in terms of the biochemical events that occur in the coupling between the β-adrenoceptor and the adenylate cyclase? Current knowledge is that the β-adrenoceptor, occupied by an agonist, activates the stimulatory GTP-binding protein (N_s), by increasing the rate of activation of N_s by guanine nucleotides.[24] The extent of N_s activation will be regulated by the characteristics of the β-adrenoceptor–agonist complex, and it determines the activation of agonist-sensitive adenylate cyclase. From the conclusions drawn in the present study, it is apparent that these characteristics are of an electronic nature. This notion leads to the concept of a conformational change of the β-adrenoceptor induced by the charge distribution on the aromatic nucleus of the *N-tert*-butylphenylethanolamines. The "activated" β-adrenoceptor will then readily interact with N_s and activate the latter protein to an extent that is dependent on the nature of the agonist.

It is attractive to speculate on the design of β_2-adrenoceptor agonists with the established equations as a rational basis. As a general rule, for high intrinsic activity, σ_m should be negative, which is easily achieved with a substituted aniline function (NHR), whereas an OH group in the para position seems to be preferred. For high affinity, the substituents should be lipophilic, with steric freedom in both meta and para positions. A combination of these

demands is found in lipophilic R substituents in the NHR function. The recent introduction of QH25 in clinical studies[25] substantiates this view to great extent. This compound was shown to be 12 times more potent than salbutamol in asthmatics.



QH25

Conclusions

Conventional QSAR analysis applied to biochemical events occurring in the coupling between the β-adrenoceptor and catalytic unit of adenylate cyclase is a valuable tool in the elucidation of the nature of these mechanisms. It is concluded that the electronic effects of aromatic substituents in the class of the *N-tert*-butylphenylethanolamines are responsible for the intrinsic activity of these derivatives, probably by the influence on charge distribution on the aromatic nucleus. Neither lipophilicity nor steric parameters seem to play a role in intrinsic activity.

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-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.[15]

β_2-Adrenoceptor Intrinsic Sympathomimetic Activity (ISA). Two concentrations of an agonist, necessary to occupy at least 95% of the β_2-adrenoceptors of the preparation described above, were calculated from its K_D value and its percentage cations at pH 8.0, according to

$$RL/B_{max} = [L]/([L] + K_D)$$

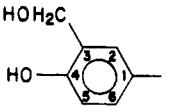
in which RL/B_{max} is the fractional receptor occupation, and [L] is the ligand concentration. These two agonist concentrations were used in the cAMP assay, described below. All assays were performed in duplicate in a final volume of 350 μL, made up of (all solutes dissolved in buffer A: 20 mM Hepes, 10 mM $MgCl_2 \cdot 6H_2O$, pH 8.0 at 20 °C) (a) 50 μL of MIX (final concentration 5×10^{-4} M), (b) 50 μL of ATP (final concentration 1×10^{-4} M), (c) 100 μL of membrane suspension (0.5 mg/mL), (d) 50 μL of GppNHp (final concentration 3×10^{-5} M), (e) 100 μL of agonist (final concentrations given in Table I) or buffer A. All reactions were carried out at 37 °C for 15 min and terminated by dilution with cold buffer B (50 mM Tris, 4 mM EDTA, pH 7.5 at 20 °C), followed by heating at 95 °C for 3 min. The cAMP content was determined by an assay kit with a cAMP binding protein (purified from bovine muscle). The difference in the cAMP production in the presence or absence of an agonist was used as a measure for the intrinsic activity of this agonist and was expressed as a fraction of the maximally observed stimulation of adenylate cyclase (thus, the intrinsic activity of salbutamol was set to 1.0).

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,[26] as previously reported.[14]

Steric Parameters. The steric branching parameter (Sb) for all substituents was calculated according to Austel et al.[27]

^{13}C NMR Chemical Shifts. ^{13}C NMR spectra (^{1}H decoupled) were recorded on a Bruker WM250 NMR spectrometer (^{13}C frequency = 62.89 MHz). All compounds were dissolved in D_2O (≈0.2 M), with $CHCl_3$ (present in a capillary centered in the NMR tube) as external standard. All solutions were acidified with HCl to a final pD value of ca. 4. The positions of the signals, combined with the information from ^{1}H coupled spectra permit the estimation of the chemical shifts (δ, in ppm, relative to Me_4Si) of each C atom present in the molecule.

As an example, the chemical shifts of the C atoms, present in the aromatic nucleus of salbutamol, are given below (s = singlet, d = doublet, mult = multiplicity, δ = coupling constant):



	δ	mult	J, Hz
C_1	131.5	s	
C_2	126.9	d	157
C_3	126.8	s	
C_4	153.9	s	
C_5	115.9	d	159
C_6	126.9	d	157

In the regression analyses, only the aromatic nucleus was considered; the differences between the measured chemical shifts and the chemical shift of the C atoms in benzene (128.5 ppm) were calculated and used as physicochemical parameters.

Electronic Parameters. Hammett's σ[28] and the *F* (inductive) and *R* (resonance) constants of Swain and Lupton[19] were taken from ref 29.

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. Regression coefficients are given with their 95% confidence intervals.

Chemistry. Compound 11, the unsubstituted *N-tert*-butylphenylethanolamine, was synthesized in our laboratories.

***N-tert*-Butyl-2-phenyl-2-hydroxyethylamine Hydrogen Chloride (11).** The free base was synthesized from 0.25 M styrene oxide and 0.25 M *N-tert*-butylamine according to a procedure as described in ref 20 and 30. The HCl salt was obtained by treating the free base with HCl/Et_2O, followed by crystallization from EtAc/MeOH (3/1, v/v): yield 33%, mp 212–213 °C. Spectral data (^{1}H NMR, IR, and MS) were in accordance with the assigned structure. Mass spectra were obtained on a Finnigan 4000 GC/MS spectrometer (70 eV). M^+ (free base): found, 193.1484; calcd, 193.1467.

Materials. The following compounds were gifts: Th 1206 (sulfate, Boehringer Ingelheim), terbutaline (sulfate, Astra), salbutamol (base, Allenburys), AH 3474 (hydrochloride, Allenburys), *N-tert*-butylnorsynephrine (base, Duphar), Du 28663 (sulfate, Duphar), SFK 56301 (base, SKF), clenbuterol (hydrochloride, Karl Thomae), and C78 (hydrochloride, UCB). The synthesis of VUF 8303 has been previously described.[14] Dihydroalprenolol, tritiated (sp act. 70 Ci/mmol), and the cyclic AMP assay kits were purchased from Amersham. All other reagents were of analytical grade.

Acknowledgment. The synthesis of compound 11 by Eric Haaksma and the aid of John C. Eriks and Frans F. de Kanter in recording the ^{13}C NMR spectra are gratefully acknowledged.

Registry No. (±)-1, 99798-66-0; (±)-2, 32550-09-7; (±)-3, 96948-64-0; (±)-4, 99798-67-1; (±)-5, 96948-66-2; (±)-6, 35763-26-9; (±)-7, 99798-68-2; (±)-8, 69930-61-6; (±)-9, 99798-69-3; (±)-10·HCl, 93739-47-0; (±)-11, 14467-31-3; (±)-11·HCl, 99798-70-6; NH_2Bu-t, 75-64-9; styrene oxide, 96-09-3; adenylate cyclase, 9012-42-4.

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