Mathematical Approach to Structure–Activity Study of Sympathomimetic Amines. Norepinephrine-Uptake Inhibition

TAKASHI BAN

Department of Pharmacology, Faculty of Medicine

AND TOSHIO FUJITA

Department of Agricultural Chemistry, Faculty of Agriculture, Kyoto University, Kyoto, Japan

Received November 4, 1968

Regression analyses by the Free and Wilson method were applied to the norepinephrine-uptake inhibition of a number of sympathomimetic amines. The original biological response parameters and their logarithms were compared in their utility for a meaningful analysis. It was demonstrated that the norepinephrine-uptake inhibition can be analyzed by constant and additive activity contributions of substituents and the parent phenethylamine skeleton. The results suggest that the conformation of the parent skeleton at the receptor site would not be changed markedly even if substituents are introduced into it as far as the compounds analyzed in this paper are concerned. Inhibitory activities of several untested compounds were predicted.

The analysis of structure–activity relationship of a series of congeneric drug molecules seems to be the first step for elucidating the mode of action as well as designing new drugs which bestow a particular merit upon the activity. Recently, regression analyses have been applied to these problems with various degrees of success.[1,2] The method of Free and Wilson, which represents the biological activity as the mathematical sum of contributions attributable to groups introduced into a common parent skeleton of congeners, is one type of such analyses.[2a]

In this paper, we wish to report the application of this method to sympathomimetic amines. Among various kinds of biological response exhibited by this class of amines, we have chosen the inhibitory activity against norepinephrine uptake in isolated rat heart. The data are taken from the careful work by Burgen and Iversen.[3] The pharmacological meaning of this action is the inhibition of inactivation and thus a potentiation of the effects of norepinephrine. Burgen and Iversen have found that norepinephrine uptake into the isolated rat heart occurs through two distinct processes and that both processes are inhibited by sympathomimetic amines. The first process (uptake 1) is operated at lower perfusion concentrations and the second (uptake 2) at higher concentrations. They have determined I_{50} concentration values of a number of sympathomimetic amines and deduced that the structural requirements for the inhibition of the two uptake processes are strikingly different from each other as well as from those of known α and β reactivity.

The biological data used for the analyses seem to meet the basic prerequisites recently suggested by Purcell and his coworkers[2c] for a meaningful application of the Free–Wilson method. As shown in Figure 1, the compounds are characterized by the presence and absence of hydroxyl, methoxyl, and methyl groups on the phenethylamine skeleton so that the structural changes among congeners are considered to be systematically gradual.

Calculations.—The regression equation according to the Free–Wilson method takes the form of eq 1, where Y represents the magnitude of the biological activity and μ represents the over-all average or activity contribution of the parent skeleton;[2a] the term a_i is the

$$Y = \sum a_i X_i + \mu \quad (1)$$

mathematical contribution to the activity of the ith substituent assigned as shown in Figure 1 where the side-chain structure is expressed by the Fischer projection. X_i takes the value of 1 or 0 depending on the presence or absence of the ith substituent at each position as shown in Tables I and II. As the biological activity parameter Y, the original inhibitory activity, A, in terms of the reciprocal of I_{50} concentration relative to phenethylamine = 100, and its logarithm, log A, were used and the two were compared in their utility for the analysis.

It was postulated that a substituent at an asymmetric carbon atom contributes differently to the total activity according to the absolute configuration. For the optically active compounds, those with known absolute configuration[4] were used for the analyses. For the racemic compounds, since half the number of molecules has the R and the other half has the S configuration at the asymmetric carbon, the value 0.5 was assigned to X_i for the substituent of each configuration.

Substitution of these values into eq 1 generates simultaneous equations, the number of which is equal to that of the compounds included in the regression analysis. For example, the uptake 1 inhibitory activity of (±)-phenylethanolamine is expressed by eq 2.

$$Y = a_3 + a_6 + {}^1/_2(a_7 + a_8 + a_9 + a_{10}) + a_{12} + a_{14} + a_{16} + \mu \quad (2)$$

Application of the restriction equations shown in Table I resulting from the summation to zero of group contributions at each position reduces the unknowns including μ from 17 to 10 for the uptake 1 inhibition. For the uptake 2 inhibition, since the optically active compounds are not included, it is impossible to know whether there is an activity difference between antipodes. In this case, the individual contributions cannot

(1) (a) C. Hansch and T. Fujita, *J. Am. Chem. Soc.*, **86**, 1616 (1964); (b) C. Hansch, E. W. Deutsch, R. N. Smith, *ibid.*, **87**, 2738 (1965).

(2) (a) S. H. Free, Jr., and J. W. Wilson, *J. Med. Chem.*, **7**, 395 (1964); (b) W. P. Purcell, *Biochim. Biophys. Acta*, **105**, 201 (1965); (c) W. P. Purcell and J. M. Clayton, *J. Med. Chem.*, **11**, 199 (1968).

(3) (a) L. L. Iversen, *Brit. J. Pharmacol.*, **25**, 18 (1965); (b) A. S. V. Burgen and L. L. Iversen, *ibid.*, **25**, 34 (1965).

(4) J. M. van Rossum, *J. Pharm. Pharmacol.*, **15**, 285 (1963).

TABLE I
OBSERVED AND CALCULATED UPTAKE 1 INHIBITORY ACTIVITY OF SYMPATHOMIMETIC AMINES[a]

Drug	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	X_{15}	X_{16}	Biological act. A Obsd	A Calcd	Log A Obsd	Log A Calcd	Antilog of log A (calcd)
(−)-Metaraminol			1	1			1			1		1	1			1	1440	775.4	3.16	2.877	754
Dopamine	1			1				1		1		1		1		1	650	472.8	2.81	2.675	452
(±)-α-Methyldopamine	1			1				1		1	0.5	0.5	0.5	0.5		1	610	596.2	2.79	2.923	838
(+)-Amphetamine			1			1		1		1		1	1			1	610	587.9	2.79	2.640	437
(±)-Hydroxyamphetamine	1					1		1		1	0.5	0.5	0.5	0.5		1	610	388.0	2.79	2.537	344
(−)-Nordefrin	1			1			1			1		1	1			1	550	866.6	2.74	3.227	1690
(−)-Norepinephrine	1			1			1			1		1		1		1	407	452.1	2.61	2.507	321
(±)-Nordefrin	1			1			0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5		1	256	405.5	2.41	2.431	269
Tyramine	1					1		1		1		1		1		1	245	264.6	2.39	2.270	186
(±)-Amphetamine			1			1		1		1	0.5	0.5	0.5	0.5		1	240	296.9	2.38	2.187	154
Metatyramine			1	1				1		1		1		1		1	215	381.6	2.33	2.305	202
(+)-Methylamphetamine			1			1		1		1	1				1		165	290.9	2.22	2.131	135
(±)-Norepinephrine	1			1			0.5	0.5	0.5	0.5		1		1		1	164	282.1	2.22	2.163	146
N-Methyldopamine	1			1				1		1		1		1	1		145	175.7	2.16	2.147	140
(−)-Epinephrine	1			1			1			1		1		1	1		110	155.0	2.04	1.999	100
Mephentermine			1			1		1		1	1		1			1	110	123.3	2.04	1.947	88.5
Phenylethylamine			1			1		1		1		1		1		1	100	173.4	2.00	1.919	83.0
(±)-Octopamine	1					1	0.5	0.5	0.5	0.5		1		1		1	85	73.9	1.93	1.777	59.8
(+)-Norepinephrine	1			1				1	1			1		1		1	79	112.1	1.89	1.818	65.8
(±)-Epinephrine	1			1			0.5	0.5	0.5	0.5		1		1	1		78	−14.9	1.89	1.654	45.1
(±)-Phenylpropanolamine			1			1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5		1	55	106.2	1.74	1.685	49.5
(−)-Ephedrine			1			1	1			1		1	1		1		50	270.2	1.70	1.983	96.2
(−)-Amphetamine			1			1		1	1					1		1	30	5.8	1.48	1.785	54.3
(+)-Phenylethanolamine			1			1	0.5	0.5	0.5	0.5		1		1		1	23	−17.3	1.36	1.427	26.7
(−)-Phenylephrine			1	1			1			1		1	1		1		20	63.9	1.30	1.648	44.5
p-Methoxyphenethylamine		1				1		1		1		1		1		1	11	2.4	1.04	1.286	19.3
(+)-Oxedrine	1					1	0.5	0.5	0.5	0.5		1		1	1		9	−223.1	0.95	1.268	18.5
(±)-Metanephrine	1				1		0.5	0.5	0.5	0.5		1		1	1		2.6	−151.2	0.42	−0.054	0.88
(+)-Normetanephrine	1				1		0.5	0.5	0.5	0.5		1		1		1	0.55	145.8	−0.26	0.454	2.8
3,4-Dimethoxyphenethylamine		1			1			1		1		1		1		1	0.55	9.1	−0.26	−0.504	0.31

[a] See Figure 1 for the substituents 1–16. Restriction equations: 4 position (substituents 1–3), $16a_1 + 2a_2 + 12a_3 = 0$; 3 position (4–6), $13a_4 + 3a_5 + 14a_6 = 0$; (*R*)-β position (7,8), $10.5a_7 + 19.5a_8 = 0$; (*S*)-β position (9,10), $5.5a_9 + 24.5a_{10} = 0$; (*R*)-α position (11,12), $4.5a_{11} + 25.5a_{12} = 0$; (*S*)-α position (13,14), $8.5a_{13} + 21.5a_{14} = 0$; *N* substituent (15,16), $9a_{15} + 21a_{16} = 0$.

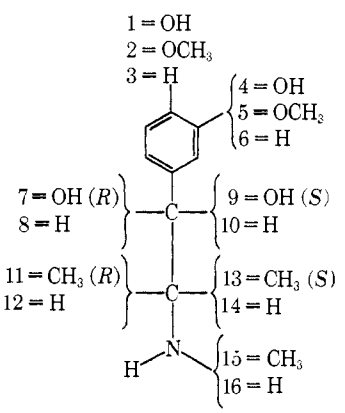

Figure 1.—Assignment of substituents.

be assigned to the groups of different configuration at the same asymmetric carbon. The assignment can be made only to the sum of the contributions of *S* and *R* substituents. Thus, if we combine the group contributions of the α or β substituents as $a_\beta(\mathrm{OH}) = a_7 + a_9$, $a_\beta(\mathrm{H}) = a_8 + a_{10}$, $a_\alpha(\mathrm{CH_3}) = a_{11} + a_{13}$, and $a_\alpha(\mathrm{H}) = a_{12} + a_{14}$, the number of unknowns is reduced from the original 17 to 13 and further to 8 by the number of restrictions, five of which are shown in Table II. The uptake 2 inhibition of (±)-metanephrine is expressed by eq 3.

$$Y = a_1 + a_5 + {}^1/_2[a_\beta(\mathrm{OH}) + a_\beta(\mathrm{H})] + a_\alpha(\mathrm{H}) + a_{15} + \mu \quad (3)$$

The simultaneous equations with ten unknowns for the uptake 1 and those with eight unknowns for the uptake 2 inhibition were then solved independently by the method of least squares. The calculations were carried out mostly by the KDC-II computer of this University.

Results and Discussion

Of 52 and 19 compounds which Burgen and Iversen originally studied for the inhibition of uptakes 1 and 2, respectively, 30 and 12 compounds of closely related structure shown in Tables I and II and Figure 1 were used for the regression analyses. The calculated activity contribution of each substituent (a_i) and of the parent skeleton (μ) are shown in Table III. These values are consistent with those conceivable from the activity enhancement factors of substituents relative to hydrogen at each position described by the original authors.[3b] They were summed up to yield the calculated total activity of each molecule. There were good correlations between the observed and calculated values, especially, when the values of log *A* were used as the biological parameter for both processes as shown in Tables I, II, and IV. The antilogarithms of the values of calculated log *A*, however, did not fit so well to the original observed activities, as expected by the correlation between their logarithms. The sum of squares of deviations between the observed and calculated activ-

TABLE II

OBSERVED AND CALCULATED UPTAKE 2 INHIBITORY ACTIVITY OF SYMPATHOMIMETIC AMINES[a]

Drug	X_1	X_2	X_3	X_4	X_5	X_6	X_β(OH)	X_β(H)	X_α(CH$_3$)	X_α(H)	X_{15}	X_{16}	Biological act. — A — Obsd	A — Calcd	Log A — Obsd	Log A — Calcd	Antilog of log A (calcd)
(±)-Metanephrine	1				1		0.5	0.5		1	1		2585	2095.9	3.41	3.55	3573
(±)-Normetanephrine	1				1		0.5	0.5		1		1	1785	1736.0	3.25	3.07	1170
(±)-Oxedrine	1					1	0.5	0.5		1	1		625	820.4	2.80	2.67	465
3,4-Dimethoxyphenethylamine		1			1			1		1		1	234	772.9	2.37	2.41	258
(±)-Epinephrine	1			1			0.5	0.5		1	1		144	438.4	2.16	2.15	140
Phenylethylamine			1			1		1		1		1	100	280.5	2.00	2.21	162
Metatyramine			1	1				1		1		1	79	−101.5	1.90	1.69	48.9
Tyramine	1					1		1		1		1	75	238.0	1.88	1.84	68.7
(±)-Amphetamine			1			1		1	0.5	0.5		1	68	68.0	1.83	1.83	68.1
2-(*p*-Methoxy)phenethylamine		1				1		1		1		1	37	−502.0	1.57	1.53	33.5
(±)-Norepinephrine	1			1			0.5	0.5		1		1	30	79.1	1.48	1.66	45.8
Dopamine	1			1				1		1		1	19	−144.0	1.28	1.32	20.7

[a] See Figure 1 for substituents 1–16. Restriction equations: 4 position (substituents 1–3), $7a_1 + 2a_2 + 3a_3 = 0$; 3 position (4–6), $4a_4 + 3a_5 + 5a_6 = 0$; β position, $2.5a_\beta$(OH) + $9.5a_\beta$(H) = 0; α position, $0.5a_\alpha(CH_3) + 11.5a_\alpha(H) = 0$; N substituent (15, 16), $3a_{15} + 9a_{16} = 0$.

TABLE III

SUBSTITUENT GROUP CONTRIBUTION[a]

Substituent	Uptake 1 inhib a_i	Uptake 1 inhib a_i'	Uptake 2 inhib a_i	Uptake 2 inhib a_i'
1 = OH	58.3	0.237	112.7	−0.041
2 = OCH_3	−269.0	−1.218	−627.3	−0.353
3 = H	−32.9	−0.113	155.2	0.331
4 = OH	110.8	0.351	−573.4	−0.569
5 = OCH_3	−25.6	−1.358	1083.4	0.838
6 = H	−97.4	−0.035	−191.4	−0.048
7 = OH	−13.4	−0.097	353.4[b]	0.543[b]
8 = H	7.2	0.052	−93.0[c]	−0.143[c]
9 = OH	−294.5	−0.682		
10 = H	66.1	0.153		
11 = CH_3	−142.2	−0.159	−407.1[d]	−0.713[d]
12 = H	25.1	0.028	17.7[e]	0.031[e]
13 = CH_3	297.0	0.516		
14 = H	−117.4	−0.204		
15 = CH_3	−207.9	−0.357	269.4	0.363
16 = H	89.1	0.153	−89.8	−0.121
Parent skeleton[f]	233.5	1.886	481.8	2.160

[a] a_i' and μ' are the logarithmic contributions. [b] Sum of contributions of groups 7 and 9. [c] Sum of contributions of groups 8 and 10. [d] Sum of contributions of groups 11 and 13. [e] Sum of contributions of groups 12 and 14. [f] μ and μ' values.

TABLE IV

CORRELATIONS BY THE REGRESSION ANALYSES

Regression eq[a]	Uptake 1 inhib n	Uptake 1 inhib s	Uptake 1 inhib r	Uptake 2 inhib[b] n	Uptake 2 inhib[b] s	Uptake 2 inhib[b] r
$A = a_iX_i + \mu$	30	209.9	0.83	12	517.0	0.93
Log $A = \Sigma a_i'X_i + \mu'$	30	0.30	0.96	12	0.22	0.98

[a] a_i' and μ' are the logarithmic contributions. [b] n is the number of compounds included in the analysis, s is the standard deviation, and r is the correlation coefficient.

ities, $\Sigma\Delta^2$, is shown in Table V. As suggested by Purcell and Clayton,[2c] the preferred choice of biological response parameter in this regression method may be the original linear data and not their logarithms.

TABLE V

THE SUM OF SQUARES OF DEVIATIONS BETWEEN OBSERVED AND CALCULATED ACTIVITIES ($\Sigma\Delta^2$)

Equation	Uptake 1 inhib n = 30	Uptake 1 inhib n = 28[a]	Uptake 2 inhib n = 12	Uptake 2 inhib n = 11[b]
$A = \Sigma a_iX_i + \mu$	881,244	339,312	1,068,801	829,680
$A = \log^{-1}(\Sigma a_i'X_i + \mu')$	1,988,149	217,953	1,385,783	409,639

[a] In $\Sigma\Delta^2$, those for (−)-metaraminol and (−)-nordefrin are not included. [b] In $\Sigma\Delta^2$, that for (±)-metanephrine is not included.

However, a close inspection of the antilogarithmic values revealed that a large part of $\Sigma\Delta^2$ comes from only a few compounds, *i.e.*, (−)-nordefrin and (−)-metaraminol for uptake 1, and from (±)-metanephrine for uptake 2 inhibition. By deleting these compounds, the values of $\Sigma\Delta^2$ were reduced by a factor of 10 for uptake 1 and a factor of 3 for uptake 2 inhibition. By the use of the original biological data, the same compounds also showed the poorest fit among others. Without these compounds, however, the values of $\Sigma\Delta^2$ were lowered only by factors of 2.5 and 1.3 and were still 1.5 and 2 times larger than those of corresponding antilogarithmic data, respectively.

In attempting to obtain more reliable group contribution values, besides (−)-nordefrin and (−)-metaraminol, (±)-normetanephrine and (±)-metanephrine,

where the value of Δ log A (obsd — calcd) exceeds 0.45, were deleted and 26 simultaneous equations with ten unknowns were once again solved for uptake 1 inhibition. The second regression analysis resulted in a higher correlation coefficient and a lower standard deviation than the first one as shown in Table VI. The value of

TABLE VI

THE STATISTICAL RESULTS OF THE SECOND ANALYSES FOR UPTAKE 1 INHIBITION

Equation	n	s	r	$\Sigma\Delta^2$
$A = \Sigma a_i N_i + \mu$	26	102.1	0.92	166,739
Log $A = \Sigma a_i N_i' + \mu'$	26	0.166	0.98	341,472[a]

[a] $\Sigma\Delta^2$ value for deviations between observed A and $\log^{-1}(\Sigma a_i' N_i + \mu')$.

$\Sigma\Delta^2$ for the antilogarithm of the calculated log A, however, was still larger than that for the calculated A. Here three-fourths of $\Sigma\Delta^2$ for the antilogarithmic data was attributed to a single compound, (±)-α-methyldopamine. Even a difference of 0.3 between log A(calcd) and log A(obsd) turns out to be a big difference of 500 between $\log^{-1}$ [log A(calcd)] and A(obsd) for a highly active compound of A(obsd) = 500, since $\log^{-1}$ [log A(calcd)] corresponds to 2A(obsd). Thus, it contributes to $\Sigma\Delta^2$ by a magnitude of 250,000. Therefore, if only a single compound of high activity does not fit very well, the total $\Sigma\Delta^2$ value for the antilogarithmic data tends to be very large. It seems that a definite preference could not be made between the original data and their logarithms for the use in the regression analysis as far as the statistical results are concerned. In this work, however, we prefer to consider that the use of logarithm of activity is justified with the exception of a few compounds, since the logarithm of activity is a free-energy-related parameter which is additive, and the use of this parameter does not predict an activity of a minus sign which is meaningless.

The reason why the above-mentioned compounds were only poorly correlated is uncertain. This may be due to an incompleteness of the model. Out of four compounds for uptake 1 and one for uptake 2 inhibition, three are those of the highest activity and the other two are of the lowest activity among the series. Further studies might reveal a difference in their mode of action from that of the others.

Thus, the present results would demonstrate that the norepinephrine uptake inhibitory activities of these amines can be analyzed by constant and additive activity contributions of substituents and the parent skeleton. The constant activity contribution of the parent skeleton would be attributable to a constant stereoelectronic requirement for the drug–receptor interaction. Thus, the conformations of the benzene ring and the amino group around the C_α–C_β axis of the phenethylamine skeleton would not be varied markedly in the drug–receptor complex even if substituents are introduced as far as the compounds analyzed in this paper are concerned. The more hydrophobic benzene ring and the positively charged amino group, with constant relative orientations may play dominant roles to fit the molecule onto the receptor sites. The conformation of the molecule on the receptor surface would differ from that conceivable in a homogeneous solution, although minor conformational changes may occur in compounds with bulky N substituents not included in the present analyses, *e.g.*, in prenylamine with $CH_2CH_2CH(Ph)_2$ and bupheuine with $CH(CH_3)CH_2CH_2Ph$. This view contrasts markedly with the one suggested by the original authors[3b] who considered that the α-methyl and β-hydroxyl groups affect the preponderant conformation of the phenethylamine skeleton so as to enhance or diminish the drug–receptor interaction.

While the constant and additive character of the hydroxyl group contribution on the benzene ring suggests that intramolecular hydrogen bonding is outweighed by the binding of each hydroxyl group to the receptor sites, those of the β-hydroxyl group do not mean necessarily that there is no interaction with the α-amino group. Since all the compounds have the α-amino group, the mathematical contributions of β-hydroxyl groups may contribute a certain amount due to the interaction.

Although some other modes of activity might be operative in the compounds of the highest and lowest activity as described above, the activity of compounds which were not tested could be predicted as far as the "normal" activity is concerned. From the logarithmic group contribution values, a_i' and μ', for the uptake 1 inhibition, α-(*S*)-3,4-dihydroxyamphetamine is estimated to have a more than 20 times greater activity than phenethylamine, and β-(3,4-dimethoxyphenyl)-β-(*S*)-hydroxy-α-(*R*)-methyl-N-methylethylamine to have the least activity in the same series of compounds. For uptake 2 inhibition, the activity of (±)-N-methyl-3-methoxy-β-hydroxyphenethylamine is estimated as approximately 200 times greater than that of phenethylamine and that of 4-methoxy-3-hydroxy-α-methylphenethylamine as below $^1/_{50}$ of that of phenethylamine. The most active compounds predicted by the use of linear group contribution values, a_i and μ, are the same as the above ones with slightly different relative activities. These predictions are consistent with the conclusions obtained by the original authors.[3b]

While the norepinephrine-uptake inhibition is one of the facets of the sympathomimetic action, the present results would indicate a possibility to elucidate the structural requirement for various biological activities exhibited by this class of pharmacologically important compounds.