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Dissociation Constants and Lipophilicity of Catecholamines and Related Compounds*

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Summary. 1. The dissociation constants (pK_{a1}, pK_{a2}, pK_{COOH}), n-octanol/water distribution coefficients D (at pH 7.4) and partition coefficients P of a series of sympathomimetic amines and of catecholamines, their precursors and metabolites were determined.

2. The influence of different substituents of compounds bearing an amino group on lipophilicity (log D and log P) and on pK_{a1} values was estimated. Introduction of a hydroxyl group reduced the lipophilicity of phenylethylamines and related compounds. Hydroxylation of the phenyl ring led to a stronger reduction of lipophilicity than did β-hydroxylation. An additional methoxy group in the molecule reduced the lipophilicity to only a very small degree. Alkylation always increased lipophilicity; the degree was dependent on the position and on the size of the alkyl group. The pK_{a1} values of phenolic amines were more influenced by the introduction of hydrophilic groups than by lipophilic groups. Hydroxylation always decreased the pK_{a1} values independent of the position of the hydroxyl group, while the introduction of a methyl group at the N-terminal of phenolic amines increased it. An α-methyl group or a methoxy group at the phenyl ring was without any effect on pK_{a1} values.

3. For noradrenaline and its precursors the following rank order of lipophilicity was observed (at pH 7.4): tyrosine > dopa > dopamine > noradrenaline; the rank order for the metabolites of noradrenaline was MOPEG > DOPEG > normetanephrine > VMA > DOMA.

4. The lipophilicity (at pH 7.4) of the metabolites of noradrenaline was correlated with the rate constants for efflux of these metabolites from peripheral organs (Graefe, 1976; Henseling et al., 1978; Fiebig and Trendelenburg, 1978a).

Key words: Dissociation constants – Lipophilicity of sympathomimetic amines – Lipophilicity of noradrenaline metabolites – Efflux of noradrenaline metabolites.

Introduction

Fiebig and Trendelenburg (1978a) perfused hearts of reserpine-pretreated rats with ^{3}H-(−)-noradrenaline and measured the rate constants for the efflux of metabolites of noradrenaline from the heart. The k-values for efflux of the different metabolites of noradrenaline decreased in the order of methoxyhydroxyphenylglycol (MOPEG) > dihydroxyphenylglycol (DOPEG) > normetanephrine (NMN) > dihydroxymandelic acid (DOMA) > methoxyhydroxymandelic acid (VMA). Henseling et al. (1978) observed the same rank order for the rate constants for efflux of these metabolites from rabbit aortic strips preincubated with ^{3}H-(−)-noradrenaline. In both studies the deaminated phenolic glycols (MOPEG and DOPEG) left the tissue with very short half times (high k-values) while the acids (DOMA and VMA) left it with considerably longer half times; the half time for the efflux of the O-methylated amine NMN was between those for the glycols and acids. The differences in the rate constants for efflux may be due to differences in the lipophilicity and/or in the degree of dissociation at physiological pH, since these metabolites belong to different chemical classes (phenolic glycols, amines, acids).

A search of the literature either failed to yield any dissociation constants of the metabolites of catecholamines, or the reported pK_a values for any given

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substance differed considerably, since different methods were used for its estimation (e.g., pK_{a1}/pK_{a2} for metanephrine = 9.52/9.74 according to Kappe and Armstrong, 1965, and 9.15/10.25 according to Sinistri and Villa, 1962). No data exist about the lipophilicity of the metabolites of catecholamines. Furthermore, little is known about the dissociation constants and the lipophilicity of the different substances which are formed during the biosynthesis of catecholamines. This study is an attempt to provide information about the lipophilicity of catecholamines, their precursors and their metabolites.

Included in this report is also the determination of the lipophilicity and the dissociation constants of a series of sympathomimetic amines. In this way it was possible to define how some chemical groups (e.g. COOH, OH, CH_3) influence the lipophilicity of these compounds.

Methods

Estimation of pK_a Values. The negative logarithms of the dissociation constants (pK_a values) of the compounds were determined by potentiometric titration as described by Albert and Serjeant (1962); and Armstrong and Barlow (1976). The individual compounds were dissolved in a known volume of bidistilled water. The water was freed from carbon dioxide and oxygen before use by boiling for 10 min and cooling in a closed vessel; the removal of these dissolved gases is necessary when readily oxidizable substances (e.g. catecholamines) are titrated and when alkali is the titrant. 20 ml of the solution (containing 0.2 mmoles of the compound) were transferred to the titration vessel of an automatic titration unit (TTT 60, autoburette ABU 13 fitted with a 4 ml burette; Radiometer Copenhagen). The titration vessel was surrounded by water circulated from a thermostat with a temperature control better than $\pm 0.1°$ C (K2R; Meßgerätewerk Lauda, FRG). A slow stream of N_2 was blown through the solution, which was stirred with a mechanically driven plastic stirrer. The pH-meter (pHM 64, combination glass electrode GK 2301 B; Radiometer Copenhagen) was usually calibrated before each titration with buffers of pH 4.008 and 9.180 at 25° C. An interval of at least 10 min was allowed for thermal equilibration (25° C) before the start of any titration and about 0.5 – 1 min was allowed after each addition of 0.2 ml standardized alkali for equilibration of the glass electrode. The alkali was KOH (0.1 M, free from carbonate; Merck Darmstadt, FRG); the molarity of KOH was checked by titration with HCl (0.1 M; Merk Darmstadt, FRG). After each addition of alkali the pH of the solution was determined and a preliminary pK_a was calculated according to the method described by Albert and Serjeant (1962). A final pK_a for each compound was obtained from the mean estimate after a complete titration. For each compound 3 – 6 final pK_a values were determined. The pK_a values listed in Table 1 are arithmetic means of these final pK_a values; they should be regarded as apparent pK_a values since they are mixed constants lying between the concentration ionization constants and the thermodynamic ionization constants. Furthermore, the pK_{a1} and pK_{a2} values of part A of Table 1 are macrodissociation constants in the case of amphoteric compounds. For these substances the first pK_a cannot be directly assigned to the amino group and the second pK_a not to the phenolic hydroxyl group. The first pK_a includes the ionization which leads to the non-charged species and the zwitterionic species; the second pK_a includes the transition of these two species into the negative charged species of the compound according to the equation:

$$^{+}HBH \underset{+H^{+}}{\overset{-H^{+}}{\rightleftharpoons}} (BH + {}^{+}HB^{-}) \underset{+H^{+}}{\overset{-H^{+}}{\rightleftharpoons}} B^{-}. \quad (1)$$

Determination of n-Octanol/Water Distribution Coefficients (D). To obtain a messure of lipophilicity at physiological pH the n-octanol/water distribution coefficients of the compounds were determined at pH 7.4. The substances were dissolved in Na^+/K^+-phosphate buffer (0.067 M, pH 7.4) saturated with n-octanol. To prevent oxidation of the compounds the buffer contained 0.01 % of Na_2SO_3 and EDTA. To one volume of the dissolved compounds 30 – 50 volumes of n-octanol saturated with phosphate buffer were added. The distribution of the compound between these two immiscible solvents was obtained by shaking the mixture in a closed tube for about 5 min (i.e., 200 inversions). This short period of shaking was used since a longer duration of shaking (e.g., 20 min for noradrenaline) did not give other results; this is in agreement with the observation of Leo et al. (1971), who reported that "about 100 inversions in roughly 5 min produce consistent results". Very vigorous shaking was avoided to prevent troublesome emulsions. After shaking the two phases were separated by centrifugation (2000 g, 15 min). The n-octanol/water distribution coefficient was obtained by analyzing the solute concentration in the water phase before and after shaking. The concentration was determined either by measuring the native fluorescence of the compounds at a wavelength of 254 – 280 nm and 286 – 320 nm (for absorption and emission, respectively) or by counting the radioactivity when radioactive substances were used. To reduce errors in the determination of distribution coefficients due to impurities of the compounds, most substances (especially the radioactively labelled ones) were purified before use by adsorption on Al_2O_3 (substances with a catechol structure) or Dowex 50 WX 4 (amines) and elution from the adsorbent with diluted HCl. For the determination of distribution coefficients concentrations of 5 – 10 µM of unlabelled substances were used, while only trace amounts of radioactive compounds were employed. Very low concentration were used to avoid the formation of aggregates in one phase.

The n-octanol/water distribution coefficient D was calculated from the following equation:

$$D = \frac{A - B}{B \cdot r} \quad (2)$$

where A is the concentration of the compound in the water phase *before* and B *after* distribution into octanol; r is the ratio of the volumes of n-octanol/buffer. For the acid deaminated metabolites of catecholamines it was not possible to determine a distribution coefficient at pH 7.4; therefore, it was measured at three different pH values (between 1.4 and 5.4). From the mean distribution coefficient of 3 – 6 determinations at any given pH the *partition* coefficient P was calculated (equation 3, see below). From these values and from the "fraction of uncharged species" present at pH 7.4 [s. equation (4)], the distribution coefficient D (at pH 7.4) was obtained.

Calculation of n-Octanol/Water Partition Coefficients (P). To obtain the customarily used partition coefficients (P) from distribution coefficients (D) (i.e., the distribution coefficients of the uncharged species of a compound) it is necessary to correct the actual concentration of the compound in the water phase for dissociation. The partition coefficient of a compound is a real measure of the lipophilic character of this compound. The "fraction of the uncharged species" (f_u) at a given pH can be calculated from the pK_a values of the compound. From f_u and D P can be calculated from the following equation (Scherrer and Howard, 1977):

$$\log P = \log D - \log f_u. \quad (3)$$

For amphoteric compounds, such as phenolic amines, the pK_a values are macrodissociation constants (see above) and therefore

only the fraction of the nondissociated and zwitterionic species can be calculated if the zwitterion constant is unknown. However, since the zwitterionic species is characterized by a net charge of zero and since its partitioning behaviour is similar to that of an uncharged species (Leo et al., 1971), it is justified to regard the concentration of *both* species as the relevant concentration; therefore the term f_u will, in the following, also be used for amphoteric compounds. For the various compounds listed in Table 1, f_u was calculated according to the following equations (a–e):

a) simple amines (compounds No. 1 and 2)

$$f_u = \frac{1}{1 + \text{antilog}\,(pK_{a1} - pH)}$$

b) phenolic amino acids (No. 7 and 13)

$$f_u = \frac{1}{1 + \text{antilog}\,(pH - pK_{COOH}) + \text{antilog}\,(pH - pK_{a2}) + \text{antilog}\,(pK_{a1} - pH)}$$

c) phenolic amines (No. 3–6, 8–12, 14–18)

$$f_u = \frac{1}{1 + \text{antilog}\,(pK_{a1} - pH) + \text{antilog}\,(pH - pK_{a2})}$$

d) phenols (No. 19, 20, 21)

$$f_u = \frac{1}{1 + \text{antilog}\,(pH - pK_{a1})}$$

e) phenolic carbonic acids (No. 22, 23, 24, 25)

$$f_u = \frac{1}{1 + \text{antilog}\,(pH - pK_{COOH}) + \text{antilog}\,(pH - pK_{a1})} \qquad (4)$$

Statistics. Results presented here are arithmetic means of the log P, log D and pK_a values. The calculation of regression lines ($y = a + b \cdot x$) and correlation coefficients (r) was carried out according to conventional procedures (Snedecor and Cochran, 1967).

Substances Used in This Study. (–)-Adrenaline hydrochloride, (–)-noradrenaline hydrochloride (Hoechst); (±)-normetanephrine hydrochloride, (±)-metaraminol hydrochloride, (±)-3–O-methylisoprenaline hydrochloride, (±)-phenylephrine hydrochloride, (±)-synephrine tartrate (C. H. Boehringer); (±)-α-methylnoradrenaline hydrochloride, (±)-metanephrine hydrochloride (Sterling Winthrop); (±)-3,4-dihydroxymandelic acid = DOMA, (±)-3,4-dihydroxyphenylglycol = DOPEG, methoxyhydroxyphenylethanol = MOPET, (±)-3-methoxytyramine hydrochloride, (±)acid = VMA (EGA-Chemie); (–)-dihydroxyphenylalanine = dopa, dihydroxyphenyl acetic acid = DOPAC, dopamine hydrochloride, (–)-tyrosine (Fluka); β-phenylethylamine hydrochloride (Roth); methoxyhydroxyphenyl acetic acid = HVA, (±)-isoprenaline hydrochloride, paratyramine hydrochloride (Serva); (±)-methoxyhydroxyphenylglycol piperazine salt = MOPEG, (±)-octopaminehydrochloride (Calbiochem; (±)-amphetamine sulphate, n-octanol (Merck). The following radioactive substances were used (all from NEN chemicals): ^{3}H-(–)-noradrenaline, ^{14}C-(±)-adrenaline, ^{3}H-dopamine, ^{3}H-(±)-metanephrine, ^{3}H-(±)-octopamine, ^{3}H-(±)-isoprenaline, ^{3}H-(±)-metaraminol, ^{14}C-phenylethylamine, ^{3}H-(±)-amphetamine sulphate; the specific activity of these compounds was between 0.04 and 30 Ci/mmol.

Results

A. Lipophilicity and Dissociation Constants of Phenylethylamines and Related Compounds

Table 1 shows the dissociation constants (pK_a values), the distribution coefficients at pH 7.4 (D) and the partition coefficients (P) of a series of phenylethylamines, phenolic amines, aromatic amino acids, catecholamines and their O-methylated and/or deaminated metabolites. The pK_a values of the carboxylic group (pK_a COOH), of the amino group and/or of the phenolic hydroxyl group (pK_{a1}, pK_{a2}) were measured by potentiometric titration at 25° C. The pK_a values of the second phenolic hydroxyl group of substances with a catechol structure are not shown, since they are not measurable by potentiometric titration. The pK_a value of the second phenolic group of catecholamines is over 12; at pH values over 12 the glass electrode does not measure correct pH values, and furthermore, catecholamines are easily oxidized (Kappe and Armstrong, 1965). The distribution coefficients D were determined in n-octanol/phosphate buffer and are shown in Table 1 as log D. Log P was calculated from the pK_a and the log D values (for details see Methods). The re-calculated values for log D and log P (last two columns in Table 1A and B) will be explained below (Section B). The compounds in Table 1A (phenylethylamines and related compounds) and in Table 1B (deaminated metabolites of catecholamines) are arranged according to their apparent lipophilicity at pH 7.4 (log D).

Of the compounds of Table 1A amphetamine and β-phenylethylamine (both substances without any hydroxyl group) showed the highest lipophilicity. The next ten substances of Table 1A are compounds with only one phenolic hydroxyl group (with the exception of isoprenaline). The differences in the lipophilicity within these ten substances are due to the methyl and/or isopropyl groups within the molecule. Compounds with two phenolic hydroxyl groups (i.e., substances with a catechol structure) showed very low lipophilicity. The β-hydroxylated catecholamine noradrenaline exhibited the lowest lipophilicity of all substances.

Of the deaminated metabolites of catecholamines (part B of Table 1) the O-methylated glycols MOPET and MOPEG exhibited the highest lipophilicity of all examined compounds (including those of part A of Table 1). The deaminated acid metabolites, on the other hand, showed the lowest lipophilicity.

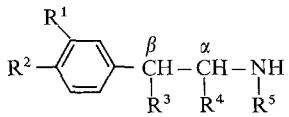
The pK_{a1} values of the compounds of Table 1A showed a certain tendency: the pK_{a1} values of non-phenolic phenylethylamines were higher than those of catecholamines.

Table 2 summarizes the influence of different substituents on the log D values, log P values and on the pK_{a1} values of the substances of Table 1A. The values for Δ log D, Δ log P (π values) and Δ pK_{a1} were obtained by comparing compounds which differ from each other by only one group.

The introduction of an hydroxyl group reduced the lipophilic nature of phenylethylamines. The degree of reduction depended on the position of the OH-group.

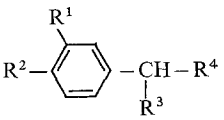
Table 1. Experimentally obtained ionization constants (pK_a values), distribution coefficients (D; determined at pH 7.4) and partition coefficients (P) of phenylethylamines and related compounds (part A) and of deaminated metabolites of catecholamines (part B). The last two columns show "recalculated" distribution and partition coefficients; a broken line indicates that this substance was used as reference (see text, Section B). The pK_a values were measured by potentiometric titration, the distribution coefficients were measured in an octanol/phosphate buffer system. Given are means of 3–6 determinations for each compound; for details see Methods

A. phenylethylamines, phenolic amines and amino acids



No.	Compound	R^1	R^2	R^3	R^4	R^5	pKa (COOH)	pK_{a1}	pK_{a2}	log D (ph 7.4)	log P	"Recalculated" log D (pH 7.4)	"Recalculated" log P
1	Amphetamine	H	H	H	CH_3	H		9.88		−0.84	1.64	−0.79	1.41
2	β-Phenylethylamine	H	H	H	H	H		9.92		−1.14	1.38	−1.14	1.07
3	O-Methylisoprenaline	OCH_3	OH	OH	H	$CH(CH_3)_2$		9.10	10.35	−1.44	0.27	−1.27	0.18
4	Metaraminol	OH	H	OH	CH_3	H		8.79	9.87	−1.68	−0.27	−1.57	−0.09
5	Isoprenaline	OH	OH	OH	H	$CH(CH_3)_2$		8.62	9.99	−1.88	−0.63	−1.66	−0.52
6	Phenylephrine	OH	H	OH	H	CH_3		8.97	10.00	−1.89	−0.31	−1.68	−0.03
7	Tyrosine	H	OH	H	COOH	H	2.3	9.37	10.64	−2.05	−2.04	−1.93	−1.79
8	Tyramine	H	OH	H	H	H		9.17	10.86	−2.12	−0.34	−2.01	−0.24
9	Synephrine	H	OH	OH	H	CH_3		9.11	10.13	−2.17	−0.45	−2.08	−0.59
10	O-Methyldopamine (Methoxy-Tyramin)	OCH_3	OH	H	H	H		9.54	10.63	−2.22	−0.08	−2.09	−0.29
11	Octopamine	H	OH	OH	H	H		8.88	9.85	−2.24	−0.75	−2.32	−0.99
12	O-Methyladrenaline (Metanephrine)	OCH_3	OH	OH	H	CH_3		9.02	10.08	−2.27	−0.64	−2.16	−0.64
13	Dihydroxyphenylalanine (Dopa)	OH	OH	H	COOH	H	2.4	8.99	10.18	−2.39	−2.38	−2.40	−2.54
14	Dopamine	OH	OH	H	H	H		8.88	10.39	−2.48	−0.99	–	–
15	O-Methylnoradrenaline (Normetanephrine)	OCH_3	OH	OH	H	H		8.82	9.91	−2.49	−1.05	−2.40	−1.04
16	Adrenaline	OH	OH	OH	H	CH_3		8.59	9.98	−2.59	−1.37	−2.55	−1.34
17	α-Methylnoradrenaline	OH	OH	OH	CH_3	H		8.55	9.65	−2.61	−1.43	−2.44	−1.40
18	Noradrenaline	OH	OH	OH	H	H		8.53	9.61	−3.01	−1.85	−2.79	−.174

B. Deaminated metabolites of catecholamines



No.	Compound	R^1	R^2	R^3	R^4	pK_a (COOH)	pK_{a1}	log D (pH 7.4)	log P	"Recalculated" log D (pH 7.4)	"Recalculated" log P
19	Methoxyhydroxyphenyl-ethanol (MOPET)	OCH_3	OH	H	CH_2OH		9.98	0.47	0.47	–	–
20	Methoxyhydroxyphenyl-glycol (MOPEG)	OCH_3	OH	OH	CH_2OH		9.50	−0.58	−0.58	−0.98	−0.54
21	Dihydroxyphenylglycol (DOPEG)	OH	OH	OH	CH_2OH		9.17	−1.01	−1.00	−1.28	−0.94
22	Methoxyhydroxyphenyl-acetic acid (HVA)	OCH_3	OH	H	COOH	4.35	10.34	−2.75	0.30	–	–
23	Dihydroxyphenylacetic acid (DOPAC)	OH	OH	H	COOH	4.53	9.84	−3.20	−0.33	−3.05	−0.10
24	Methoxyhydroxymandelic acid (VMA)	OCH_3	OH	OH	COOH	3.44	9.93	−4.61	−0.65	−4.20	−0.71
25	Dihydroxymandelic acid (DOMA)	OH	OH	OH	COOH	3.47	9.53	−4.63	−0.70	−4.50	−1.11

Table 2. Influence of substitutens on the lipophilicity and on pK_{a1} values of phenylethylamines and related compounds. The substituent constants (Δ log values) and the Δ pK_{a1} values were obtained by comparing log *D*, log *P* and pK_{a1} values of compounds which differ from each other by only one substituent. The factors (i.e. antilogarithms of the Δ log values) indicate changes in lipophilicity due to the introduction of the substituent. Given are means (± S.E.) for Δ log values or geometric means for the factors

Introduced	Substituent (position)[a]	Compared compounds[b]	Δ log *D* (factor)	Δ log *P* (πvalue) (factor)	Δ pK_{a1}
OH	R^2	2/8, 6/16, 4/17	−0.87 ± 0.09 (0.135)	−1.31 ± 0.21 (0.049)	−0.31 ± 0.07[c] (0.75)
	R^1	7/13, 8/14, 11/18 9/16	−0.47 ± 0.10 (0.339)	−0.75 ± 0.17 (0.178)	−0.39 ± 0.05
	R^3	8/11, 10/15, 14/18	−0.31 ± 0.12 (0.490)	−0.75 ± 0.17 (0.178)	−0.45 ± 0.13
OCH_3	R^1	8/10, 11/15, 9/12	−0.15 ± 0.05 (0.708)	−0.08 ± 0.17 (0.832)	+0.07 ± 0.15
COOH	R^4	8/7, 14/13	+0.08 ± 0.01 (1.202)	−1.55 ± 0.15 (0.028)	+0.16 ± 0.05
CH_3	R^5	18/16, 15/12 11/9	+0.24 ± 0.10 (1.738)	+0.40 ± 0.05 (2.512)	+0.16 ± 0.05
	R^4	18/17, 2/1	+0.35 ± 0.05 (2.239)	+0.34 ± 0.08 (2.188)	−0.01 ± 0.03
	$-O-R^1$ (phenolic OH, meta)	14/10, 18/15, 16/12, 5/3	+0.39 ± 0.06 (2.455)	+0.70 ± 0.12 (5.012)	+0.47 ± 0.08
$CH(CH_3)_2$	R^5	18/5, 15/3	+1.09 ± 0.06 (12.30)	+1.27 ± 0.07 (18.62)	+0.19 ± 0.13

[a] Compare chemical structure in Table 1A

[b] For numbers see Table 1A

[c] Shown is only the mean difference between the pK_{a1} values of the compounds 6/16 and 4/17, since the difference between the pK_{a1} values of the non-phenolic amines 2/8 was much more pronounced

Hydroxylation in para position (R^2) led to a very pronounced reduction in lipophilicity (by a factor of 7.4 and 20.4 for log *D* and log *P*, respectively), whereas hydroxylation in meta position (R^1) or at the β-C atom (R^3) reduced the lipophilicity to a smaller degree (by a factor of 2 – 3 and 5 – 6 for log *D* and log *P*, respectively). Hydroxylation of the C-atom in the side chain of deaminated metabolites of catecholamines (of Table 1B) led to a comparatively more pronounced reduction of the lipophilicity, namely by a factor of 28.2 (log *D*) or 10.2 (log *P*) (not shown in Table 2). The introduction of a methoxy group in meta position (R^1) of phenylethylamines led to only a very small reduction of lipophilicity. A carboxyl group at the α-C atom (amino acids) reduced the partition coefficient (log *P*) but increased the apparent lipophilicity (log *D*). Alkylation always increased lipophilicity; the degree again depended on the position and also on the size of the alkyl group. N-methylation (R^5) and α-methylation (R^4) had about the same effect, whereas O-methylation of the phenolic hydroxyl group in meta position (R^1) increased lipophilicity to a higher degree (by a factor of 2.4 and 5 for log *D* and log *P*, respectively). O-methylation of the deaminated metabolites of catecholamines (see Table 1B) increased lipophilicity by a facto of 2 and 2.5 for log *D* and log *P*, respectively (not shown in Table 2). The introduction of an isopropyl group at the N-atom (R^5) of the compounds of Table 1A increased log *D* by a factor of about 12, whereas a methyl group in the same position increased it only by a factor of about 1.7. A similar influence of alkylation and β-hydroxylation on the lipophilicity of non-phenolic phenylethylamines was recently reported by Mihailova and Testa (1978).

The pK_{a1} values of the phenolic amines of Table 1A were more influenced by the introduction into the molecule of hydrophilic than of lipophilic groups. Hydroxylation decreased the pK_{a1} values independ of the position of the OH-group; this decrease was more pronounced when a hydroxyl group was first introduced into a non-phenolic amine, e.g. β-phenylethylamine. The carboxyl group of aromatic amino acids led to an increase of the pK_{a1} values. The introduction of a α-methyl group or a methoxy group

Table 3. Influence of deamination of catecholamines on lipophilicity at pH 7.4. Given are mean values; for details see legend to Table 2

Amine	Deaminated metabolite	Δ log D	Change in lipophilicity at pH 7.4 (factor)
Dopamine or methoxytryptamine	glycol	+2.69	489.8
	acid	−0.63	0.234
Noradrenaline or normetanephrine	glycol	+1.96	91.2
	acid	−1.87	0.013
Adrenaline or metanephrine	glycol	+1.64	43.7
	acid	−2.19	0.006

and methylation of the phenolic OH-group in meta position did virtually not influence the pK_{a1} values of phenolic amines. However, the introduction of a methyl or isopropyl group at the N-terminal of phenolic amines increased the pK_{a1} values.

Table 3 shows the effect of deamination of catecholamines on log D. Deamination of catecholamines or of their O-methylated metabolites leads to glycols which were 44 to 490 times more lipophilic than the original amine; the deaminated acids of the corresponding amines, on the other hand, were less lipophilic than the amines (by a factor of 4 to 155).

B. "Recalculated" log D and log P Values

By means of the Δ log P values of Table 2, and with the help of the experimentally obtained log P values of any given compound it was possible to recalculate log P for all the compounds according to the equation of Hansch et al. (1962): $\log P_i = \log P_o + \Delta \log P_x$; (where $\log P_o$ = log partition coefficient of a reference compound, o, and $\log P_i$ = log partition coefficient of a derivative, i, which differs from o by the substituent X; when D is substituted for P, the same equation can be used to calculate log D_i).

When dopamine or the deaminated metabolites of dopamine were used as reference substances, the recalculated values of log D and log P (Table 1) were very similar to the experimentally obtained values. The mean difference between these two kinds of values in Table 1A was very small: -0.016 ± 0.043 (log P) and 0.112 ± 0.017 (log D; $n = 17$ each). When β-phenylethylamine was used as the reference compound, the difference between the experimentally obtained and the recalculated values was more pronounced: the mean difference for log P was 0.305 ± 0.040 and for log D was 0.093 ± 0.018 ($n = 17$ each).

C. Correlation Between the Rate Constants for Efflux of the Metabolites of Noradrenaline and Lipophilicity

When the rate constants (log k values) for efflux of the O-methylated and the deaminated metabolites of noradrenaline from perfused hearts (rat heart: Fiebig and Trendelenburg, 1978a; rabbit and cat heart: Graefe 1976) were plotted against the log D values (taken from Table 1) a significant correlation was obtained (Fig. 1). A correlation with about the same slope was observed between the log k values for efflux of noradrenaline

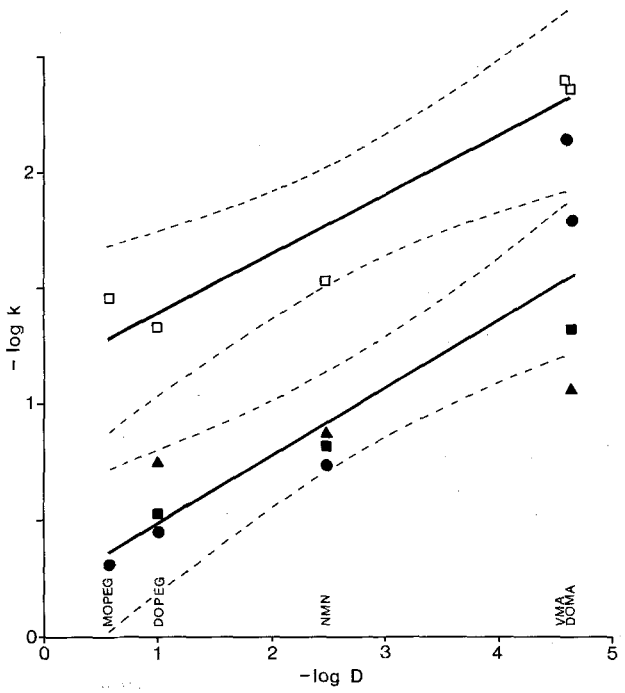


Fig. 1. Correlation between the mean rate constants for efflux and the distribution coefficients of the metabolites of noradrenaline. Ordinate: negative logarithm of the k values for efflux of the metabolites (min^{-1}); abscissa: negative logarithm of the distribution coefficients at pH 7.4 of the metabolites. Closed symbols: rate constants for efflux of the metabolites from perfused hearts, (●) rat heart (values taken from Fiebig and Trendelenburg, 1978a), (▲) cat heart and (■) rabbit heart (taken from Graefe, 1976). Open symbols: rate constants for the efflux of the metabolites of noradrenaline from rabbit aortic strips (taken from Henseling et al., 1978). Upper regression line: $y = 1.1317 + 0.2551\ x$, $S_a = 0.1485$, $S_b = 0.0468$, $r = 0.9530$, $n = 5$, $P < 0.05$; lower regression line: $y = 0.1987 + 0.2915\ x$, $S_a = 0.1833$, $S_b = 0.0586$, $r = 0.8563$, $n = 11$, $P < 0.001$, broken lines indicate confidence limits at the 95% level

metabolites from rabbit aortic strips (Henseling et al., 1978) and the log D values of these compounds (Fig. 1).

Since the apparent lipophilicity (log D) of a compound is greatly dependent on the degree of dissociation at pH 7.4 (i.e., $\log D = \log f_u + \log P$; see Methods), it was of interest to find out whether log k is also linearly related to log f_u or log P. While no significant correlation was found between log k and log P, the correlation of log k on log f_u was highly significant (aorta: $y = a + bx = 1.337 + 0.2535x$, $S_a = 0.0795$, $S_b = 0.0308$, $r = 0.9785$, $n = 5$, $P < 0.01$; heart: $y = 0.4790 + 0.275x$, $S_a = 0.1364$, $S_b = 0.0548$, $r = 0.8589$, $n = 11$, $P < 0.001$).

Discussion

The compounds formed within the biosynthesis of catecholamines show considerable differences in the apparent lipophilicity at physiological pH of 7.4. At this pH tyrosine is the most lipophilic compound followed by dopa, dopamine and noradrenaline. The differences in the lipophilicity at pH 7.4 are due to a different lipophilicity of the uncharged molecule (see log P values in Table 1) and also to a different degree of dissociation at pH 7.4, which in turn depends on the pK_a values. The amino acid tyrosine, the first substance in the biosynthesis of catecholamines, exhibits a relatively high lipophilicity at pH 7.4. This is not only because tyrosine has only one hydroxyl group, but this is also due to the fact that pH 7.4 is not far away from the isoelectric point (i.e. the average of the pK_{aCOOH} and the pK_{a1}; about 5.83), where the major species of tyrosine is the isoelectric form. Since, at the isoelectric point, the water solubility of polyprotic amphoteric compounds like amino acids is lowest (Peck and Benet, 1978), the apparent lipophilicity of such compounds is presumably highest at pH values around the isoelectric point. Also Leo et al. (1971) reported that amphoteric molecules such as amino acids are most lipophilic when they contain an equal number of positive and negative charges. This holds also true for the amino acid dopa, the next product in the biosynthesis of catecholamines. However dopa already shows a smaller lipophilicity at pH 7.4 than does tyrosine; the additional phenolic hydroxyl group considerably reduces the lipophilicity. Dopamine, the first catecholamine in the biochemical pathway, is less lipophilic at pH 7.4 than the amino acid dopa, but is more lipophilic than noradrenaline and adrenaline. Finally, noradrenaline shows the lowest lipophilicity (at pH 7.4) of all compounds formed in the biosynthesis of catecholamines.

Degradation of catecholamines by monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT) leads to metabolites which are either more or less lipophilic at pH 7.4 than are their parent substances. O-methylation increases the lipophilicity only by a factor of 2–3. However, the glycols, formed from catecholamines by MAO and aldehyde reductase, are much more lipophilic than are catecholamines. At pH 7.4 these glycols are even more lipophilic than is β-phenylethylamine. The acid deaminated metabolites of catecholamines, on the other hand, formed by MAO and aldehyde dehydrogenase, are much more hydrophilic at pH 7.4 than are the catecholamines. A similar ore even higher degree of hydrophilicity at pH 7.4 would be expected for the sulphates of the glycols, since an attempt to measure the octanol/buffer distribution coefficient of the sulphate of methoxy hydroxyphenylglycol at pH 7.4 was unsuccessful (Mack and Bönisch, unpublished results).

The differences in lipophilicity at pH 7.4 (measured by means of the distribution coefficients D at pH 7.4) are obviously the reason for the different rate coefficients (k values) for the efflux of the metabolites of noradrenaline from incubated or perfused isolated *peripheral* tissues. (This conclusion does not hold true for the efflux of the acid metabolites and the sulphated glycols of catecholamines from certain regions of the *central* nervous system, since high rate constants for the efflux of these substances from the central nervous system were observed, which are obviously due to carrier-mediated processes; Meek and Neff, 1972.) The significant correlation observed between -log D and -log k (see Fig. 1) was mainly due to the relative degree of dissociation of these metabolites at pH 7.4; this is especially valid for the acid metabolites. At physiological pH only a very small part of the acid metabolites is present in the undissociated form and, therefore, able to penetrate biological membranes.

When isolated tissues are incubated or perfused with noradrenaline, most of the acid metabolites are recovered from the tissue, and only very small amounts escape into the incubation of perfusion fluid (Levin, 1974; Graefe, 1976; Henseling et al., 1978; Fiebig and Trendelenburg, 1978a). This phenomenon can be explained by the high degree of dissociation of the acid metabolites. On the other hand, for the glycols the degree of dissociation does not play an important role for their ability to penetrate membranes, since log D and log P values for these substances do not differ much. It is of interest to note that the ratio mentioned above is then reversed: the major part of the glycols is recovered from the incubation or perfusion fluid, but very little from the tissue.

The permeability coefficient of a substance for passage across a given biological membrane is a) directly proportional to its distribution coefficient between membrane and water and b) inversely proportional to its molecular weight (Lieb and Stein, 1969),

unless a carrier-mediated transport or, for an electrolyte, a specific pore is involved in the transfer of the molecule. Hence, for mass corrected molecules or for molecules having similar molecular weights (like the metabolites of noradrenaline) a double logarithmic graph of the rates of permeation versus distribution coefficients should result in a regression line with a slope of unity (Lieb and Stein, 1969), provided the system used for the determination of distribution coefficients reflects the partitioning behavior of the membrane. Figure 1 shows such a correlation between the logarithms of the rate constants for efflux of the metabolites of noradrenaline from peripheral tissues and the logarithms of the distribution coefficients of these substances determined in the system octanol/water (pH 7.4). However, the slope of the regression line of Fig. 1 is less than unity, indicating that the octanol/water system does not accurately reflect the partitioning of the metabolites of noradrenaline in the biological membranes involved in the efflux of these substances.

It should be mentioned that the values shown in Fig. 1 do not ideally lie on the regression line; therefore, it cannot be excluded that, beside the apparent lipophilicity, additional factors contributed to the experimentally observed rate constants for efflux of the metabolites of noradrenaline. For example, it would be conceivable that a small part of the efflux of the amine normetanephrine from peripheral tissues is carrier-mediated, since normetanephrine is a good substrate for the extraneuronal uptake process. Uhlig et al. (1976), however, failed to observe an inhibition of the efflux of ^{3}H-normetanephrine from the perfused rat heart by corticosterone which, on the other hand, inhibited the uptake of ^{3}H-normetanephrine.

Recently it has been found for the perfused rat heart that catecholamines and phenethylamines are subject not only to saturable neuronal and/or extraneuronal uptake, but also to a non-saturable uptake (noradrenaline: Fiebig and Trendelenburg, 1978b; isoprenaline: Bönisch, 1978; phenylephrine: Rawlow, Kurahashi, Trendelenburg, unpublished results). Apparently, this non-saturable uptake is more pronounced for highly than for poorly lipophilic amines, since the rate constants were higher for isoprenaline and phenylephrine than for noradrenaline (for log D values, see Table 1). Moreover, the uptake of the most lipophilic phenethylamine, amphetamine, is known to be non-saturable (Thoenen et al., 1968); apparently, for a very highly lipophilic phenethylamine, non-saturable, lipophilic uptake can be so high as to entirely mask the carrier-mediated (cocaine-sensitive) neuronal uptake.

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