Title: Characterization of the species-specific acid-base equilibria of adrenaline and noradrenaline

Authors: Arash Mirzahosseini, Tamás Pálla, Gábor Orgován, Gergő Tóth, Béla Noszál

PII: S0731-7085(18)32819-X
DOI: https://doi.org/10.1016/j.jpba.2019.02.035
Reference: PBA 12503

To appear in: *Journal of Pharmaceutical and Biomedical Analysis*

Received date: 14 December 2018
Revised date: 20 February 2019
Accepted date: 20 February 2019

Please cite this article as: Mirzahosseini A, Pálla T, Orgován G, Tóth G, Noszál B, Characterization of the species-specific acid-base equilibria of adrenaline and noradrenaline, *Journal of Pharmaceutical and Biomedical Analysis* (2019), https://doi.org/10.1016/j.jpba.2019.02.035

TITLE PAGE

Characterization of the species-specific acid-base equilibria of adrenaline and noradrenaline

AUTHORS

Arash Mirzahosseini, Tamás Pálla, Gábor Orgován, Gergő Tóth, Béla Noszál*

Department of Pharmaceutical Chemistry, Semmelweis University, Budapest, Hungary

Research Group of Drugs of Abuse and Doping Agents, Hungarian Academy of Sciences, Hungary

*to whom correspondence should be addressed:

Prof. Béla Noszál

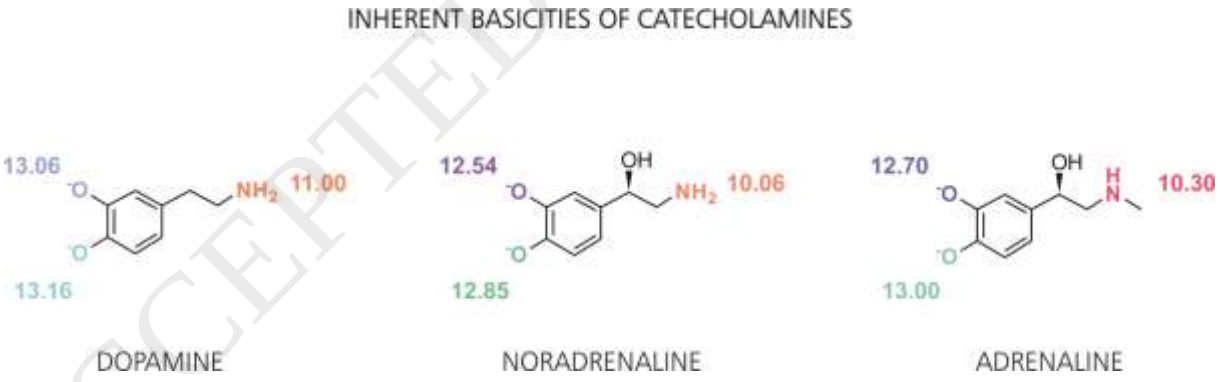
Department of Pharmaceutical Chemistry, Semmelweis University

H-1092 Budapest, Hőgyes E. u. 9, Hungary

Phone/Fax: +3612170891

e-mail: noszal.bela@pharma.semmelweis-univ.hu

GRAPHICAL ABSTRACT



HIGHLIGHTS

- complete set of protonation constants determined for adrenaline and noradrenaline
- ^{1}H NMR method and auxiliary compounds used to determine microscopic constants
- protonation species populations reflect physicochemical behavior

ABSTRACT

Adrenaline, noradrenaline, the biogenic catecholamines of vital importance, and four closely related compounds were studied by ^{1}H NMR-pH titrations, and the resulting acid-base properties are quantified in terms of three macroscopic and twelve microscopic protonation constants for both molecules. The species-specific basicities are interpreted by means of inductive and shielding effects by comparing the protonation constants of the catecholamines, including dopamine. The site-specific basicities determined this way could be key parameters for the interpretation of biochemical behavior.

KEY WORDS

catecholamine; epinephrine; pK_a; microspeciation; NMR-pH titration

1. INTRODUCTION

Adrenaline and noradrenaline are biogenic catecholamines of vital importance. The transport and biochemical reactions of these neurotransmitters in the central nervous system are influenced by the site-specific acid-base properties and the concomitant number and location of charges. Both compounds contain one amino and two phenolate sites as basic centers; the gross charge of these compounds therefore varies between -2 and +1. Both studied molecules exist in eight different site-specific protonation states in aqueous solution. The analytical and physico-chemical properties of these compounds have been extensively studied since the 1950's; the acid-base parameters have been reported in fourteen scientific papers [1-14], three of which contain site-specific protonation constants [5-7]. There is, however, a dearth of a reliable and complete set of microscopic protonation constants in the literature, and a great deal of uncertainty exists regarding the protonation constants, especially that of the first protonation step. This latter issue is mostly due to an important shortcoming of the reported studies: the lack of unbiased pH calibration, which is a typical source of errors in basic media. The first protonation process of these compounds takes place at a very high pH range in aqueous media.

The main objective of our work was to determine the protonation macro- and microconstant of adrenaline and noradrenaline. Since the two phenolates and the amino sites protonate in a highly overlapping fashion, microscopic protonation constants are crucial to elucidate the protonation pathways. In order to determine accurate protonation constants we used NMR-pH titrations with unbiased, referenced pH calibration and appropriate evaluation methods, including studies on closely related auxiliary compounds.

Table 1. The protonation constants of adrenaline reported in the literature. The method of analysis is depicted as Pot for potentiometry, UV for UV-VIS spectrophotometry, NMR for ^{1}H NMR spectroscopy. The macroscopic and microscopic protonation constants in the table are depicted as defined in the Results section.

Ref	Method	logK_1	logK_2	logK_3	logk_O^O	logk_O^N	logk_{ON}^O	logk_{OO}^N
1	Pot	-	9.9	8.71	-	-	-	-
1	UV	-	-	8.88	-	-	-	-
2	Pot	13	9.95	8.66	-	-	-	-
3	Pot/UV	-	9.89	8.75	-	-	-	-
4	Pot	11.99	10.04	8.52	-	-	-	-
5	NMR	-	10.5	9.3	10.4	9.9	9.9	9.4
6	Pot	-	10.49	9.23	-	-	-	-
6	NMR	-	10.47	9.25	10.37	9.76	9.96	9.35
7	Pot/UV	13.1	9.84	8.64	9.81	9.1	9.38	8.67
8	Pot	13.15	9.87	8.63	-	-	-	-
9	Pot	12.1	10.01	8.74	-	-	-	-

Table 2. The protonation constants of noradrenaline reported in the literature. Abbreviations and remarks are identical with those in Table 1.

Ref	Method	logK_1	logK_2	logK_3	logk_O^O	logk_O^N	logk_{ON}^O	logk_{OO}^N
1	Pot	-	9.78	8.73	-	-	-	-
1	UV	-	-	8.9	-	-	-	-
2	Pot	13	9.7	8.64	-	-	-	-
10	Pot	-	9.98	8.82	-	-	-	-
11	Pot	11.13	9.73	8.57	-	-	-	-
12	Pot	-	9.64	8.62	-	-	-	-
4	Pot	11.56	9.59	8.73	-	-	-	-
5	NMR	-	-	-	10.1	9.9	9.7	9.5
13	Pot	-	9.93	8.57	-	-	-	-
7	Pot/UV	12.9	9.53	8.58	9.42	9.00	9.11	8.69
14	Pot	-	9.78	8.82	-	-	-	-

Table 1 and 2 contain the protonation constants reported for adrenaline and noradrenaline [1-14]. The data show that the most dubious value is logK_1, which fluctuates in value between ca. 11 and 13. The main reason for this disparateness is that the determination of protonation constant values higher than log$K$≈12 requires special considerations when it comes to pH determination. With a ^{1}H NMR-pH titration method using *in situ* pH indicators optimized for strongly basic media [15-16] we can determine the first macroscopic protonation constant with greater certainty. Using NMR spectroscopy also has the added benefit of being insensitive to oxidation of the compounds and the carbonate error of glass electrode. The microscopic protonation constants available in the literature are incomplete; therefore in the present work we elucidate the complete microspeciation scheme of adrenaline and noradrenaline using closely related auxiliary model compounds for some minor microspecies, and the case-tailored deductive method presented for dopamine in our recent paper [17].

2. MATERIALS AND METHODS

2.1 Materials

Adrenaline, noradrenaline and the model compounds, metanephrine, normetanephrine were purchased from Sigma-Aldrich, normacromerine was obtained from Alfa Aesar. All other reagents were of analytical grade. The deionized water was prepared with a Milli-Q Direct 8 Millipore system.

2.2 NMR spectroscopy measurements

All NMR measurements were carried out on a Varian Unity Inova DDR spectrometer (599.9 MHz for 1H) with a 5 mm inverse-detection gradient (IDPFG) probehead at 25 °C. The solvent was an aqueous solution with H_2O:D_2O, 95:5, V/V% (1.0 mol·dm^{-3} ionic strength), using DSS (sodium 3-(trimethylsilyl)-1-propanesulfonate) as the chemical shift reference compound. The sample volume was 600 μL, titrand and pH indicator concentrations were ca. 5 mmol·dm^{-3}. In ^{1}H NMR experiments pH values were determined by internal indicator molecules optimized for NMR [15-16], and the water resonance was suppressed by a double pulse field gradient spin echo sequence (number of transients = 16, number of points = 16384, acquisition time = 3.33 s, relaxation delay = 1.5 s). The ^{1}H NMR

indicators used at 1.0 mol·dm^{-3} ionic strength were imidazole (pH range 5.5-8.9 and >13), sarcosine (pH range 8.7-11.8), and acetone oxime (pH range 11.4-13.1).

2.3 Data analysis

For the analysis of NMR titration curves (proton chemical shifts versus pH), the software Origin Pro 8 (OriginLab Corp., Northampton, MA, USA) was used. In all regression analyses the non-linear curve fitting option was used with the following function [18]:

$$(1) \quad \delta_{obs(pH)} = \frac{\delta_L + \sum_{i=1}^{n} \delta_{H_iL} 10^{\log \beta_i - ipH}}{1 + \sum_{i=1}^{n} 10^{\log \beta_i - ipH}}$$

where δ_L is the chemical shift of the unprotonated ligand (L), δ_{H_iL} values stand for the chemical shifts of successively protonated ligands, where n is the maximum number of protons that can bind to L, and β is the cumulative protonation macroconstant. The standard deviations of logβ values from the regression analyses were used to calculate the Gaussian propagation of uncertainty to the other equilibrium constants derived in the Results chapter.

3. RESULTS

The macro- and microscopic protonation schemes of adrenaline and noradrenaline can be seen in Figure 1. The top line indicates macroequilibria with the stoichiometry of the successively protonated ligand and the K_1, K_2, K_3 stepwise macroscopic protonation constants. The microspeciation scheme is depicted below with the eight microspecies and their one-letter symbols (a, b … h), and the 12 microscopic protonation constants (k^{pO}, k_{pO}^{N}, k_{pO}^{mO} …). The superscript of the microscopic protonation constants (k) indicates the protonating group, while the subscript (if any) shows the site(s) already protonated. The pO, mO and N symbolize the para-phenolate (at position 1 of the aromatic ring),

meta-phenolate (at position 2 of the aromatic ring) and amino sites, respectively. Some examples of the macro- and microconstants of catecholamines are:

(2) $$K_1 = \frac{[HL^-]}{[L^{2-}][H^+]} \qquad K_2 = \frac{[H_2L]}{[HL^-][H^+]} \qquad K_1K_2 = \beta_2 = \frac{[H_2L]}{[L^{2-}][H^+]^2}$$

(3) $$k^{pO} = \frac{[b]}{[a][H^+]} \qquad k^{N}_{pO} = \frac{[f]}{[b][H^+]} \qquad k^{mO}_{pON} = \frac{[h]}{[f][H^+]}$$

Concentrations of the various macrospecies comprise the sum of the concentration of those microspecies that contain the same number of protons, for example:

(4) $$[L^{2-}] = [a]$$

(5) $$[HL^-] = [b] + [c] + [d]$$

(6) $$[H_2L] = [e] + [f] + [g]$$

(7) $$[H_3L^+] = [h]$$

Equations (8)-(10) show the relevant relationships between the micro- and macroconstants to our calculations [19]:

(8) $$K_1 = k^{pO} + k^{mO} + k^{N}$$

(9) $$K_1K_2 = k^{pO}k^{mO}_{pO} + k^{pO}k^{N}_{pO} + k^{mO}k^{N}_{mO} = k^{mO}k^{pO}_{mO} + k^{N}k^{pO}_{N} + k^{N}k^{mO}_{N}$$

(10) $$K_1K_2K_3 = k^{pO}k^{mO}_{pO}k^{N}_{pOmO} = k^{mO}k^{pO}_{mO}k^{N}_{pOmO} = \ldots$$

Equation (10) can be written in six different, equivalent ways depending on the path of protonation. For the determination of all microconstants, the introduction and utilization of auxiliary compounds are necessary.

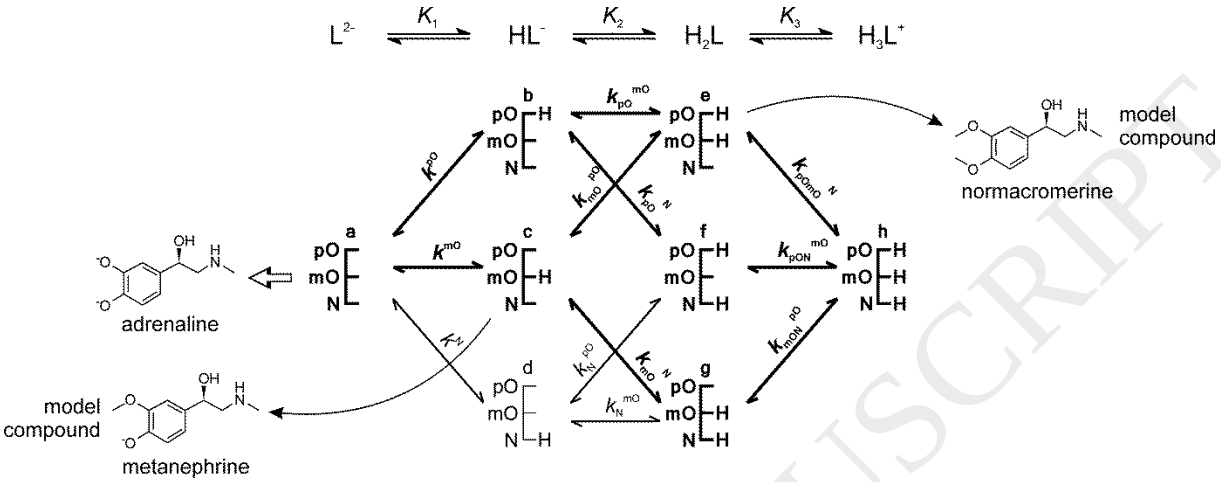


Figure 1. The protonation macro- and microequilibrium schemes of adrenaline. In the microspeciation scheme components of the major protonation pathways are in bold. The two model compounds, metanephrine and normacromerine are also indicated. The protonation scheme of noradrenaline is completely analogous to that of adrenaline.

Since the microspeciation schemes of adrenaline and noradrenaline are analogous, the deductive method used will be described here for adrenaline only. First, the NMR-pH titration of adrenaline afforded the three macroscopic protonation constants (logK_1, logK_2, logK_3). Concerning the two phenolate sites, their separate, specific basicities can not be determined by spectroscopic methods only, since no any spectroscopic parameter is selective of any of these sites. Therefore derivative compounds and deductive method had to be applied. The protonation of normacromerine (the 3,4-dimethoxy derivative of adrenaline) models the adrenaline protonation step from microspecies e to microspecies h, thus the determined constant was introduced as $\log k^{N}_{pOmO}$: 9.627 ±0.009. The replacement of phenolic –OH groups by methoxy groups does not perturb the amino protonation significantly, as seen in the case of dopamine [17]. The titration of metanephrine (the 3-methoxy derivative of adrenaline), whose most basic form models microspecies c, afforded the two

macroscopic protonation constants: 10.253 ±0.004 and 9.332 ±0.007. Using an analogous relationship to equation (10) with the macroscopic protonation constants of adrenaline and metanephrine, logk^{mO} was calculated as follows:

$$\log k^{mO} = (\log K_1 + \log K_2 + \log K_3) - (10.253 + 9.332) - 0.216 \quad (11)$$

Te correction factor (-0.216) for the methylation of only one phenolate moiety was taken from the recent work on dopamine [17]. As seen from the protonation constants of catechol and guaiacol [17], the introduction of a methyl group on the dihydroxyphenyl moiety perturbs the real protonation constant by 0.216 log units. Subsequently, the value of $\log k_{mO}^{pO}$ could be calculated with the help of the macroconstants of metanephrine:

$$\log k_{mO}^{pO} = (10.253 + 9.332) - \log k_{pOmO}^{N} - 0.216 \quad (12)$$

The value of k_{mO}^{N} can be calculated using an equation analogous to equation (8) for metanephrine:

$$k_{mO}^{N} = 10^{10.253} - k_{mO}^{pO} \bullet 10^{0.216} \quad (13)$$

The value of $\log k_{mO}^{pO}$ can be calculated as:

$$\log k_{mO}^{pO} = \log K_{1_{MTNP}} + \log K_{2_{MTNP}} - \log K_{1_{NRMCM}} \quad (14)$$

where indices MTNP and NRMCM are abbreviations of metanephrine and normacromerine, respectively.

Subsequently, the interactivity parameter between the pO and N moieties could also be calculated:

(15) $\Delta\log E_{\mathrm{pO-N}} = \log k^{\mathrm{N}}_{\mathrm{mO}} - \log k^{\mathrm{N}}_{\mathrm{pOmO}}$

Finally, by using the interactivity parameters from dopamine [17] all the remaining microscopic protonation constants could be calculated with equations analogous to (15). The interactivity parameter is considered to be an invariant quantity in analogous moieties of different compounds [20]. The pO/mO interactivity parameter (between the two phenolates) can be taken directly from dopamine. The macroscopic and microscopic protonation constants and the interactivity parameters of adrenaline and noradrenaline are compiled in Table 3 and Table 4, respectively.

Table 3. The macroscopic and microscopic protonation constants (25 °C, 1.0 mol·dm^{-3} ionic strength), and interactivity parameters of adrenaline in log units ±s.d.

$\log K_1$	13.022 ±0.005	$\log k^{\mathrm{pO}}$	13.00 ±0.03	$\log k_{\mathrm{pO}}{}^{\mathrm{mO}}$	9.44 ±0.04
$\log K_2$	10.172 ±0.003	$\log k^{\mathrm{mO}}$	12.70 ±0.02	$\log k_{\mathrm{mO}}{}^{\mathrm{pO}}$	9.74 ±0.02
$\log K_3$	8.879 ±0.005	$\log k^{\mathrm{N}}$	10.30 ±0.05	$\log k_{\mathrm{pO}}{}^{\mathrm{N}}$	9.99 ±0.04
$\log\Delta E_{\mathrm{pO/mO}}$	3.26 ±0.02	$\log k_{\mathrm{pOmO}}{}^{\mathrm{N}}$	9.627 ±0.009	$\log k_{\mathrm{N}}{}^{\mathrm{pO}}$	12.69 ±0.04
$\log\Delta E_{\mathrm{pO/N}}$	0.32 ±0.03	$\log k_{\mathrm{pON}}{}^{\mathrm{mO}}$	9.08 ±0.06	$\log k_{\mathrm{mO}}{}^{\mathrm{N}}$	9.95 ±0.03
$\log\Delta E_{\mathrm{mO/N}}$	0.36 ±0.04	$\log k_{\mathrm{mON}}{}^{\mathrm{pO}}$	9.42 ±0.04	$\log k_{\mathrm{N}}{}^{\mathrm{mO}}$	12.35 ±0.05

Table 4. The macroscopic and microscopic protonation constants (25 °C, 1.0 mol·dm^{-3} ionic strength), and interactivity parameters of noradrenaline in log units ±s.d.

$\log K_1$	13.02 ±0.01	$\log k^{\mathrm{pO}}$	12.85 ±0.03	$\log k_{\mathrm{pO}}{}^{\mathrm{mO}}$	9.27 ±0.05
$\log K_2$	9.75 ±0.01	$\log k^{\mathrm{mO}}$	12.54 ±0.05	$\log k_{\mathrm{mO}}{}^{\mathrm{pO}}$	9.59 ±0.03
$\log K_3$	8.73 ±0.02	$\log k^{\mathrm{N}}$	10.06 ±0.07	$\log k_{\mathrm{pO}}{}^{\mathrm{N}}$	9.74 ±0.06
$\log\Delta E_{\mathrm{pO/mO}}$	3.26 ±0.02	$\log k_{\mathrm{pOmO}}{}^{\mathrm{N}}$	9.379 ±0.009	$\log k_{\mathrm{N}}{}^{\mathrm{pO}}$	12.53 ±0.04
$\log\Delta E_{\mathrm{pO/N}}$	0.32 ±0.03	$\log k_{\mathrm{pON}}{}^{\mathrm{mO}}$	8.92 ±0.06	$\log k_{\mathrm{mO}}{}^{\mathrm{N}}$	9.70 ±0.06
$\log\Delta E_{\mathrm{mO/N}}$	0.36	$\log k_{\mathrm{mON}}{}^{\mathrm{pO}}$	9.27	$\log k_{\mathrm{N}}{}^{\mathrm{mO}}$	12.18

	±0.04		±0.04		±0.06

4. DISCUSSION

A submolecular (i.e. site-specific) acid-base characterisation of catecholamines allows deeper insight into highly specific biochemical processes compared to the macroscopic pK_a values [21]. It is generally accepted that the protonation state of the ligand is of cruical importance in the activation of the receptors, as it has been described previously for adrenergic receptors [22-24]. The first protonation step of catecholamines can be quantified by a macroconstant of 13.02, which is in agreement with some of the literature reports [2,7,8]. The first protonation step is mostly composed of the parallel protonations of the two phenolate groups, however the protonation of the para-phenolate is slightly more favoured, due to the activating ortho-para directing effect of the alkyl chain. The amino moiety protonates only with two orders of magnitude lower probability in the first step. The second and third protonation steps of adrenaline and noradrenaline involve the parallel and overlapping protonation of the remaining basic moieties; therefore the second and third macroscopic constants are much closer to one another in value. Figure 2 shows that following the protonation of one of the phenolates, the amino group is slightly more favoured in the second step compared to the remaining phenolate. The basicity of the amino group is influenced modestly only by the protonation of the phenolates, contrary to the two phenolates, which interact with each other strongly (as shown by the interactivity parameter values). The large pO/mO interactivity parameter causes the two successive phenolate protonations to fall relatively far from one another on the pH scale; this phenomenon is best explained by the presence of an intramolecular hydrogen bond between the –OH and $–O^-$ in the monoprotonated catechol part. It is a peculiarity of the catecholamines that almost all of the microspecies are involved in the major protonation pathways.

By comparing the three catecholamines studied (dopamine, noradrenaline, adrenaline), one can understand the subtle differences in the inherent basicities of the three basic moieties. As seen on the Graphical Abstract, the presence of the hydroxyl group in noradrenaline exerts an electron withdrawing effect, thereby decreasing the inherent basicities of all three basic moieties relative to dopamine. This is in correlation with the covalent distance between the hydroxyl group and the basic

moieties, as follows. There are three covalent bonds to the amino group, decreasing its basicity by 1 log unit, while the four covalent bonds to the meta phenolate group drops its basicity by 0.5 log units, and the five covalent bonds to the para phenolate group reduces its basicity by 0.3 log units. Another effect of the hydroxyl group is the shielding that occurs between the amino group and the two phenolates, as this manifests in lower pO/N and mO/N interactivity parameter values in noradrenaline and adrenaline relative to dopamine. The shielding capacity of the hydroxyl is able to somewhat diminish the electron-withdrawing effect of protonation, provided that the neighboring basic moiety is situated on the opposite side of the hydroxyl. Note, that the interactivity parameter between the two phenolates can be assumed to be constant across the three catecholamines, because these two moieties are uninterrupted in their relative position to one another. The additional methyl group on the adrenaline amino group is expected to have a positive electronic effect, thereby increasing the inherent basicity of the amino relative to noradrenaline. This is surely the case, as the presence of the methyl group results in approximately 0.3 log unit increase in the inherent basicity of the amino moiety. Furthermore, even the phenolate inherent basicities are increased by 0.15 log units, due to the methyl group, and the electron-transmitting effects of the hydroxyl and the aromatic ring.

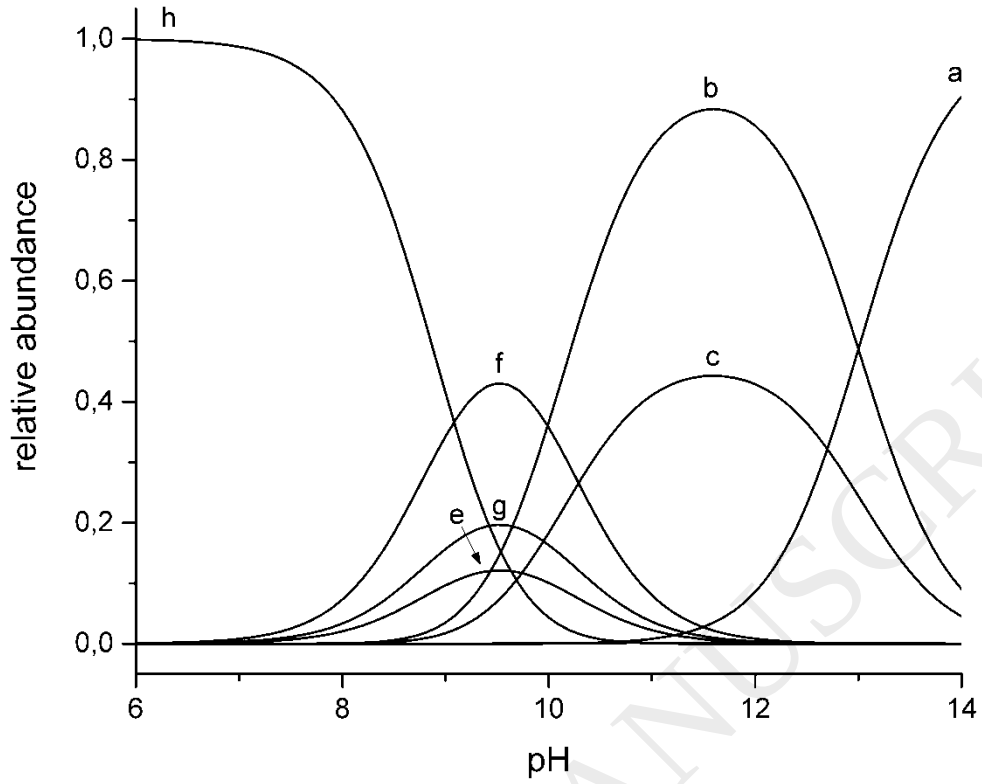


Figure 2. The relative abundance of the adrenaline microspecies as a function of pH. Microspecies d almost merges into the baseline.

5. CONCLUSIONS

The twelve species-specific, and three macroscopic protonation constants as well as the three interactivity parameters determined for both adrenaline and noradrenaline, are important datasets for the understanding of the behaviour of these biologically important compounds. The acid-base properties of the studied compounds were determined with greater accuracy by using NMR spectroscopy and *in situ* pH indicators optimized for strongly basic media. It can be confirmed from the microspeciation schemes that catecholamines occur overwhelmingly in the fully protonated state near neutral pH, bearing nearly +1 gross charge in both compounds. This charge is needed for the receptor binding [25]; however, this charge poses a serious obstacle for membrane penetration, and hinders the pharmacokinetic features of these compounds.

ACKNOWLEDGEMENT

This research was supported by the ÚNKP-17-4-I-SE-80 and FIKP-2018 (A.M.)/ÚNKP-17-4-I-SE-66 (G.T.) New National Excellence Program of the Ministry of Human Capacities of Hungary. The authors report no conflict of interest.

REFERENCES

1. Lewis GP. The importance of ionization in the activity of sympathomimetic amines, *Brit J Pharmacol*, **1954**, *9*, 488-493.
2. Jameson RF, Neillie WFS. Complexes formed by adrenaline and related compounds with transition-metal ions. Part I. Acid dissociation constants of the ligands. *J Chem Soc*, **1965**, *0*, 2391-2395.
3. Kappe T, Armstrong MD. Ultraviolet absorption spectra and apparent acidic dissociation constants of some phenolic amines. *J Med Chem*, **1965**, *8*, 368-374.
4. Grgas-Kužnar B, Simeon VI, Weber OA. Complexes of adrenaline and related compounds with Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+}. *J Inorg Nucl Chem*, **1974**, *36*, 2151-2154.
5. Granot J. NMR studies of catecholamines. Acid dissociation equilibria in aqueous solutions. *FEBS Lett*, **1976**, *67*, 271-275.
6. Jameson RF, Hunter G, Kiss T, A. ^{1}H nuclear magnetic resonance study of the deprotonation of *L*-dopa and adrenaline. *J Chem Soc, Perkins Trans 2*, **1980**, *0*, 1105-1110.
7. Gergely A, Kiss T, Deák Gy, Sóvágó I. Complexes of 3,4-dihydroxyphenyl derivatives IV. Equilibrium studies on some transition metal complexes formed with adrenaline and noradrenaline. *Inorg Chim Acta*, **1981**, *56*, 35-40.
8. Jameson RF, Kiss T. The oxovanadyl(IV) catalysed oxidation of adrenaline by molecular oxygen. *J Chem Soc Dalton Trans*, **1986**, *0*, 1833-1838.
9. Gerard C, Chehhal H. Stabilite de complexes metalliques avec des ligands d'interet biologique: La dopamine et l'adrenaline. *Bull Soc Chim Fr*, **1997**, *134*, 1069.
10. Andrews AC, Lyons TD, O'Brien TD. Copper (II), and nickel (II) complexes of various antihistaminic compounds: Their stability and thermodynamic values. *J Chem Soc*, **1962**, *0*, 1776-1780.
11. Rajan KS, Davis JM, Colburn RW. Metal chelates in the storage and transport of neurotransmitters: Interactions of metal ions with biogenic amines. *J Neurochem*, **1971**, *18*, 345-364.

12. Antikainen PJ, Witikainen U. A comparative study on the ionization of catechol amines in aqueous solutions. *Acta Chem Scand*, **1973**, *27*, 2075-2082.
13. Rajan KS, Skripkus A, Marks GE, Davis JM. Coordination of biogenic amines with synaptosomal and vesicular metal ions: Equilibrium and spectral studies in modal systems. *Bioinorg Chem*, **1976**, *6*, 93-117.
14. Sandmann BJ, Luk HT. A potentiometric study of lithium complexation with catecholamines. *J Pharm Sci*, **1986**, *75*, 73-77.
15. Szakács Z, Hägele G, Tyka R. 1H/31P NMR pH indicator series to eliminate the glass electrode in NMR spectroscopic pKa determinations, *Anal Chim Acta*, **2004**, *522*, 247–258.
16. Orgován G, Noszál B. Electrodeless, accurate pH determination in highly basic media using a new set of ^{1}H NMR pH indicators, *J Pharm Biomed Anal*, **2011**, *54*, 958-964.
17. Mirzahosseini A, Pálla T, Orgován G, Tóth G, Noszál B. Dopamine: Acid-base properties and membrane penetration capacity. *J Pharm Biomed Anal*, **2018**, *158*, 346-350.
18. Szakács Z, Noszál B. Protonation microequilibrium treatment of polybasic compounds with any possible symmetry, *J Math Chem*, **1999**, *26*, 139–155.
19. Bjerrum N. Dissoziationskonstanten von mehrbasischen Säuren und ihre Anwendung zur Berechnung molekularer Dimensionen, *Physik Chem Stoechiom Verwandschaftsl*, **1923**, *106*, 219-241.
20. Santos MA, Esteves MA, Vaz MC, Frausto da Silva JJRF, Noszál B, Farkas E. Microscopic acid-base equilibria of a synthetic hydroxamate siderophore analog, piperazine-1,4-bis(N-methylacetohydroxamic acid), *J. Chem. Soc. Perkin Trans 2*, **1997**, *10*, 1977-1983.
21. Tóth G, Baska F, Schretner A, Rácz Á,. Noszál B. Site-specific basicities regulate molecular recognition in receptor binding: in silico docking of thyroid hormones, *Eur Biophys J*, **2013**, *42*, 721-730.
22. Ghanouni P, Schambye H, Seifert R, Lee TW, Rasmussen SGF, Gether U, Kobilka KB. The effect of pH on 2 adrenoceptor function: Evidence for protonation-dependent activation. *J Biol Chem*, **2000**, *275*, 3121-3127.

23. Butista DL, Morris DH, Stein L, Asher W, Hammitt T. A two model receptor system of the alpha$_{1D}$ adrenergic receptor to describe interactions with epinephrine and BMY7378. *J Chem Inf Model*, **2006**, *46*, 334-344.

24. Dror RO, Arlow DH, Maragakis P, Mildorf TJ, Pan AC, Xu H, Borhani DW, Shaw DE. Activation mechanism of the 2-adrenergic receptor. *PNAS*, **2011**, *108*, 18684-18689.

25. Trumpp-Kallmeyer S, Hoflack J, Bruinvels A, Hibert M. Modeling of G-protein-coupled receptors: application to dopamine, adrenaline, serotonin, acetylcholine, and mammalian opsin receptors. *J Med Chem*, **1992**, *35*, 3448-3462.