European Journal of Pharmaceutical Sciences 47 (2012) 921–925

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Thyroxine lipophilicity is dominated by its zwitterionic microspecies

Károly Mazák *, Gergő Tóth, József Kökösi, Béla Noszál

Semmelweis University, Department of Pharmaceutical Chemistry, Research Group of Drugs of Abuse and Doping Agents, Hungarian Academy of Sciences, Hőgyes E. u. 9., H-1092 Budapest, Hungary

ARTICLE INFO

Article history:
Received 3 August 2012
Received in revised form 17 September 2012
Accepted 18 September 2012
Available online 28 September 2012

Keywords:
Zwitterion lipophilicity
Microscopic partition coefficient
Microscopic protonation constant
Thyroid hormones
Thyroxine
Deductive method

ABSTRACT

Species-specific partition coefficients were determined for a triprotic molecule for the first time. Thyroxine, the vitally important thyroid hormone which exists in solution in the forms of eight microspecies due to its phenolate, amino and carboxylate basic sites, was studied by combined methods of microspeciation and lipophilicity. Partition of the individual microspecies was mimicked by model compounds of the closest possible similarity, then correction factors were determined and introduced. The non-charged microspecies is only 2.40 times as lipophilic as its zwitterionic protonation isomer, showing that for thyroxine the iodinated aromatic rings are the structural elements that determine the lipophilicity of this molecule, and the protonation state of the other substituents plays only a minor role. The overwhelming dominance of the zwitterionic form, however, ensures that its contribution to the overall lipophilicity exceeds 14,500 times that of the non-charged one. This fact is so far the sharpest counter-example of the widespread belief that passive diffusion into lipophilic media is predominated by the non-charged species. The lipophilicity profile of thyroxine is expressed, calculated and depicted in terms of species-specific lipophilicities over the entire pH range.

1. Introduction

Thyroxine (3,5,3′,5′-tetraiodothyronine, T4), liothyronine (3,5,3′-triiodo L-thyronine, T3) and "reverse" liothyronine (3,3′,5′-triiodo L-thyronine, rT3), the thyroid hormones are formed in the human thyroid gland by iodination and coupling reactions of tyrosine (Chemburkar et al., 2010). T3 and rT3 are produced during the peripheral metabolism primarily when T4 is converted to T3 or rT3 (Nagao et al., 2011). The thyroid hormones are general enhancers of cellular metabolism. They are also crucial for the normal development of the central nervous system (CNS) in infants, the skeletal growth in children, and also for the normal function of multiple organ systems in adults (Cody, 1980).

Thyroid hormones are very lipophilic substances, they are therefore able to cross the plasma membrane of target cells even by passive diffusion. Nevertheless, many active iodothyronine transporters have been identified (e.g. the OATP1C1, OATP14 organic anion transporter and the amino acid transporter LAT-1), and it is now accepted that the cellular uptake is effected by energy dependent, carrier-mediated processes as well (Hennemann et al., 2001).

Lipophilicity is a molecular property of immense importance in pharmacy, bio-, and medicinal chemistry. Its applications include apparently diverse fields such as drug design for targeted delivery and development of chromatographic separations. The ability of drugs to diffuse passively through biological membranes has long been known to be largely influenced by their lipophilicity (Fujita, 1990). The pH-partition hypothesis postulates that absorption of ionizable drugs takes mainly place in compartment(s) where the local pH ensures the maximum concentration of the non-charged form relative to the ionized form(s) (Avdeef, 2002). In addition, lipophilicity is a tool to unravel biologically relevant intramolecular interactions and intermolecular forces of recognition (Testa et al., 1996; Liu et al., 2011).

In order to quantitate lipophilicity, the commonly accepted parameter is log *P*, the logarithm of the partition coefficient. It is the concentration ratio of a solute in a single electrical state, being in equilibrium between two immiscible solvents. Octanol is the most often used organic solvent, and the octanol–water partition coefficient is the most widely used descriptor of lipophilicity in QSAR studies (Hansch, 1994). When more than one electrical species are present in solution, the observed ratio of concentrations is the distribution coefficient (*D*), a pH-dependent, overall parameter, composed of the intrinsic lipophilicity of the various electrical species present (p_i), and their mole fractions in the aqueous phase (x_i).

$$D = \sum (x_i p_i) \quad (1)$$

The variation of log *D* as a function of the aqueous pH is the lipophilicity profile. It is a *sine qua non* condition to understand the pharmacokinetic, toxicokinetic and even pharmacodynamic properties (Pagliara et al., 1997).

* Corresponding author. Tel./fax: +36 1 2170891.
E-mail address: mazak.karoly@pharma.semmelweis-univ.hu (K. Mazák).

0928-0987/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejps.2012.09.009

The lipophilicity of ionizable drugs and solutes has been underrepresented in the literature, due mainly to the lack of reliable methods to determine the partition coefficients of the ionic forms. This is especially true for ionization/protonation isomers, such as the zwitterionic and non-charged forms of amphoteric compounds.

In order to gain insight into the partition microequilibria of amphoteric drugs at the species-specific level, we have recently elaborated a method and studied on three systems (Mazák et al., 2011; Mazák and Noszál, 2012a,b). The partition properties of the compound in question and its microspecies-mimicking synthetic derivatives were investigated on niflumic acid, a highly lipophilic non-steroidal anti-inflammatory drug. We reported, for the first time for any compound, experimental microscopic partition coefficients for the two protonation isomers (Mazák et al., 2011). Subsequently we reported the complete set of experimental microscopic partition coefficients of morphine, the best known opiate alkaloid. The lipophilicity profile of morphine was expressed, calculated and depicted in terms of species-specific lipophilicities over the entire pH range (Mazák and Noszál, 2012a). Finally we reported the complete set of experimental microscopic partition coefficients of the amphoteric eburnane alkaloid *cis*- and *trans*-apovincaminic acids, providing the first experimental proof for the predominant contribution of zwitterionic species to the overall lipophilicity (Mazák and Noszál, 2012b).

As expected, the non-charged form was much more lipophilic than its zwitterionic protonation isomer in each case. Although niflumic acid is a dominantly zwitterionic compound, having 16 times as many zwitterionic than non-charged microspecies in aqueous solution, because of the orders of magnitude larger lipophilicity of the non-charged form, its contribution to the overall lipophilicity is around 25 times more important than that of the zwitterionic protonation isomer.

The non-charged microspecies of the cis-apovincaminic acid is 30,900 times as lipophilic as its zwitterionic protonation isomer, while the analogous ratio for the trans-epimer is around 15,800. Due to the overwhelming dominance of the zwitterionic form, however, its contribution to the overall lipophilicity exceeds eight and five times that of the non-charged one for the two epimers, respectively.

A recent study on the microscopic acid–base properties of these triprotic thyroid hormones showed that in aqueous solutions they exist in their differently ionized forms (Tóth et al., 2012), and the concentration of the non-charged, most lipophilic form is several magnitudes smaller for each molecule.

Because of the very poor aqueous solubility of thyroxine, only one experimental study (Comer and Box, 2008) attempted to characterize the lipophilicity of the molecule in the octanol–water system. The reported log *P* 3.21 value, however, is still to be clarified, whether it refers to the lipophilicity of the non-charged microspecies or all the neutral forms collectively.

In this study we characterize the lipophilicity of the microspecies of thyroxine (T4), and quantitate their contribution to the overall lipophilicity. Experimental microscopic partition coefficients for triprotic molecules have not been reported before.

2. Materials and methods

2.1. Materials

L-Thyroxine sodium salt was obtained from Sigma–Aldrich Co. All other reagents were of analytical grade (Reanal). All solutions were prepared from freshly boiled distilled water.

2.2. Synthesis of derivatives with reduced number of basic site(s)

The carboxymethyl (C-methyl) ester of T4 was synthesized according to literature applying direct esterification of D-tyrosine (Ishigami et al., 2009). The O-methyl ether of T4 was synthesized according to literature involving methylation with diazomethane and subsequent alkaline hydrolysis of the O-methyl-carboxymethyl ester (Loeser et al., 1938). The structure of the synthesized compounds was confirmed by ^{1}H NMR spectra in DMSO-d_6, using a Varian Inova 600 MHz NMR spectrometer.

2.3. Partition coefficient measurements by the stir-flask method

The distribution coefficients were calculated from the absorbance of the molecules before and after partitioning at several octanol/water phase ratios (Mazák et al., 2003). We usually had to use the octanol phase for absorbance measurements because of the poor water solubility of these molecules. The total concentration of the measured molecules was 1.1×10^{-4} mol/dm^3 before partitioning. The partitioning was performed in a room thermostated to 25 °C. The absorbance measurements were done in another room, at ambient temperature. For the pH control buffers composed of phosphate and citrate; and standardized HCl and NaOH solutions were used with an ionic strength of 0.15 M. For pH-measurement a Metrohm 6.0234.110 combined glass electrode, calibrated by aqueous NIST standard buffer solutions, was used in the pH range 4–8.

3. Results

3.1. Acid–base equilibria

Thyroxine contains two acidic and one basic site, thus it exists in solutions in eight microscopic protonation forms (microspecies), and 12 microconstants are needed to describe its protonation microequilibria (Bjerrum, 1923; Noszál, 1990).

The protonation scheme of thyroxine is depicted in Fig. 1.

A recent study of our research group (Tóth et al., 2012) has presented the microconstants and the logarithmic distribution

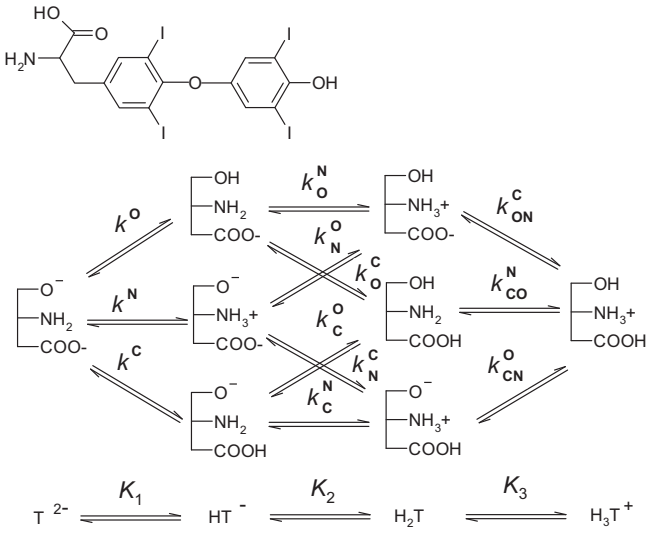

Fig. 1. The micro- and macro-speciation scheme of thyroxine, where microconstants with superscript O, N and C belong to the phenolate, amino and carboxylate site, respectively, and K_1, K_2 and K_3 are stepwise macroconstants (log K_1 = 8.60, log K_2 = 6.59, log K_3 = 2.01 from Tóth et al., 2012). The superscript on the microconstant indicates the protonating site, while the subscript (if any) stands for the site already protonated.