Journal of Pharmaceutical and Biomedical Analysis 76 (2013) 112–118

Contents lists available at SciVerse ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Species-specific lipophilicity of thyroid hormones and their precursors in view of their membrane transport properties

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

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

ARTICLE INFO

Article history:
Received 26 October 2012
Received in revised form
10 December 2012
Accepted 10 December 2012
Available online 20 December 2012

Keywords:
Thyroid hormone
Lipophilicity
Microscopic partition coefficient
Membrane transport
Liothyronine
Log *p*

ABSTRACT

A total of 30 species-specific partition coefficients of three thyroid hormones (thyroxine, liothyronine, reverse liothyronine) and their two biological precursors (monoiodotyrosine, diiodotyrosine) are presented. The molecules were studied using combined methods of microspeciation and lipophilicity. Microspeciation was carried out by ^{1}H NMR-pH and UV-pH titration techniques on the title compounds and their auxiliary derivatives of reduced complexity. Partition of some of the individual microspecies was mimicked by model compounds of the closest possible similarity, then correction factors were determined and introduced. Our data show that the iodinated aromatic ring system is the definitive structural element that fundamentally determines the lipophilicity of thyroid hormones, whereas the protonation state of the aliphatic part plays a role of secondary importance. On the other hand, the lipophilicity of the precursors is highly influenced by the protonation state due to the relative lack of overwhelmingly lipophilic moieties. The different log *p* values of the positional isomers liothyronine and reverse liothyronine represent the importance of steric and electronic factors in lipophilicity. Our investigations provided clear indication that overall partition, the best membrane transport – predicting physico-chemical parameter depends collectively on the site-specific basicity and species-specific partition coefficient. At physiological pH these biomolecules are strongly amphipathic due to the lipophilic aromatic rings and hydrophilic amino acid side chains which can well be the reason why thyroid hormones cannot cross membranes by passive diffusion and they are constituents of biological membranes.

The lipophilicity profile of thyroid hormones and their precursors are calculated and depicted in terms of species-specific lipophilicities over the entire pH range.

1. Introduction

Thyroid hormones 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) are 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 [1]. These hormones are formed in the human thyroid gland by iodination and coupling reactions of tyrosine [2,3]. T4 is formed upon coupling of two diiodotyrosine (DIT) molecules while T3 and rT3 by coupling DIT with monoiodotyrosine (MIT). T3 and rT3 are produced during the peripheral metabolism primarily when T4 is converted to T3 or rT3 [4]. The structure of the thyroid hormones and their precursors are in Fig. 1.

Thyroid hormones are the only known molecules in the human body that contain iodine, the heaviest element in the body. The covalently bonded iodine is an electron withdrawing substituent that reduces the basicity of every protonating group, decreases the water-solubility and increases the solubility in apolar organic solvents. Thyroid hormones are therefore highly lipophilic molecules due to the iodinated aromatic rings. In spite of their lipophilicity, the cellular uptake of thyroid hormones is effected by energy dependent, carrier-mediated processes [5]. Moreover, iodothyronines are normal constituents of biological membranes [6]. The hormones are oriented towards the center of the bilayer with the phenolic end of the molecule and they rigidify the membranes [7]. The obvious reason for it is that these hormones are preferably amphipathic with lipophilic aromatic rings and hydrophilic amino acid side chains.

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 pH-partition hypothesis postulates that absorption of ionizable drugs mainly takes place in compartment(s) where the local pH ensures the maximum concentration of the non-charged form relative to the ionized form(s) [8]. In addition, lipophilicity is an important

* Corresponding author. Tel.: +36 06 1 217 0891; fax: +36 06 1 217 0891.
E-mail address: noszal.bela@pharma.semmelweis-univ.hu (B. Noszál).

0731-7085/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jpba.2012.12.010

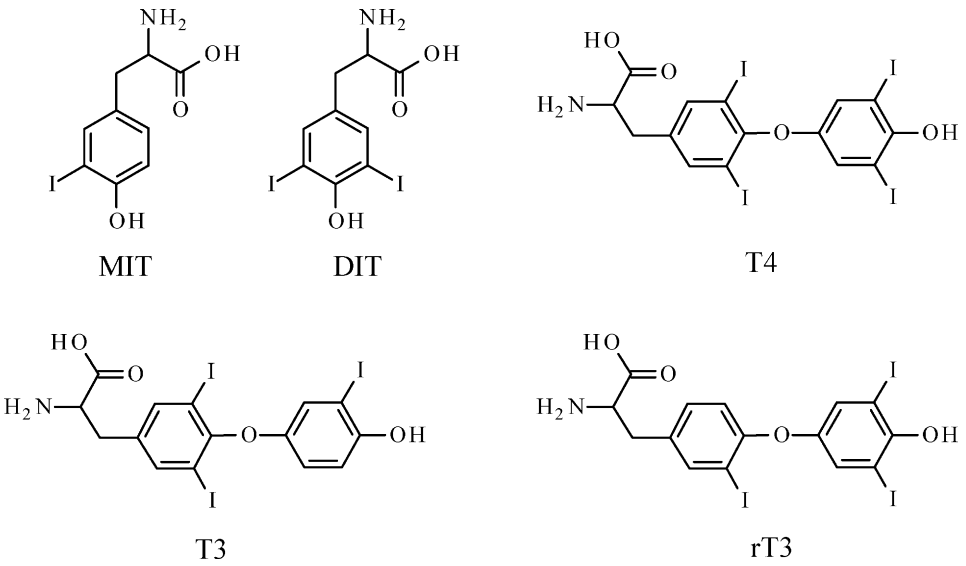

Fig. 1. Constitutional formulas of the thyroid hormones and their precursors studied.

physicochemical parameter affecting the affinity of a molecule for lipophilic binding sites [9]. In order to quantitate lipophilicity, the commonly accepted parameter is log *P*, the logarithm of the partition coefficient. It is the logarithm of the equilibrium concentration ratio of a solute between two immiscible solvents. The log *P* value can be defined for macrospecies in any electrical state. Octanol is the most often used organic solvent and the octanol–water partition coefficient is the most frequently used descriptor of lipophilicity in QSAR studies [10]. 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 [11].

The lipophilicity of ionizable drugs, especially the ionization/protonation isomers, such as the zwitterionic and non-charged forms of amphoteric compounds has been underrepresented in the literature, mainly due to the lack of reliable methods to determine the partition coefficients of the ionic forms. Our research group elaborated and used a method to determine the species-specific partition microequilibria of amphoteric drugs [12]. We reported, for the first time for any compound, experimental microscopic partition coefficients for the two protonation isomers. We also proved experimentally that zwitterions can be predominant in membrane penetration of drugs [13].

In our recent study the site-specific microspeciation of thyroid hormones (T4 and T3) and their precursors (MIT, DIT and Tyr) was reported [14]. We assumed that the different basicities of the phenolate group in the different compounds are the distinctive conditions for their biochemical processes. At physiological pH the phenolic hydroxyl group of MIT and T3 exists approximately in 15% in the deprotonated form whilst the analogous value of DIT and T4 is approximately 90% due to an additional iodine atom on the ring. This difference could be the reason for the different biological role of the two precursors (MIT vs. DIT) and hormones (T4 vs. T3).

The species-specific partition coefficients of T4 were also reported [15]. We determined that – surprisingly enough – the non-charged microspecies is only a bit more lipophilic than the zwitterionic protonation isomer and the overwhelming dominance of the zwitterionic form ensures that its contribution to the overall lipophilicity exceeds 14,500 times that of the non-charged one. No literature data were found regarding the octanol–water partition coefficients of other investigated compounds. Hillier reported that at physiological pH the "partition coefficient" between phosphatidylcholine and the aqueous environment was 12,000 for T4 and 22,000 for T3 [16]. Dickson et al. reported values of 17,500 and 23,500 for T4 and T3, respectively, between lipid and aqueous phases in multilamellar liposomes [17]. Hillier has also investigated the pH dependence of the binding to the phospholipide membranes. Thyroid hormones bind to phoshatidylcholine to the greatest extent at pHs where the molecules are in zwitterionic forms [16]. These data also unambiguously show that for the interpretation of membrane transport processes of thyroid hormones the macroscopic log *P* values are not sufficient, a profound insight needs the knowledge of the partition coefficients of the individual ionic forms as well.

Here we report the lipophilicity of the microspecies of all the thyroid hormones and their precursors. Their contribution to the overall lipophilicity is characterized and quantitated. Using these values the role of the species-specific lipophilicity of thyroid hormones in their membrane transport could be clarified. The role of the presence and position of iodine atom in the partition coefficients is also explained.

2. Materials and methods

2.1. Materials

L-diiodotyrosine, L-monoiodotyrosine, L-thyroxine, L-liothyronine, 3,3′,5′-triiodo L-thyronine (rT3), HPLC grade 1-octanol and DMSO-d_6 were obtained from Sigma–Aldrich Co. Other chemicals of analytical grade were purchased from commercial suppliers and are used without further purification. All solutions were prepared from bidistilled Millipore water.

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

The synthesis of the carboxymethyl (C-methyl) esters and the *O*-methyl ethers of the investigated compounds was described in