

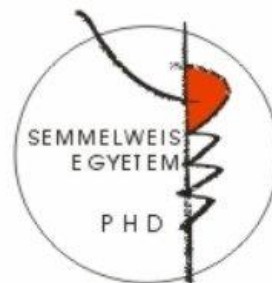
Site- and species-specific hydrolyses rates of ester-type drugs

Theses of doctoral (PhD) dissertation

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1. Introduction

Ester group is an abundant unit in bio- and drug molecules, and also, in illicit drugs of abuse. Some of the prime examples are: acetyl-choline, membrane-forming phosphatidyl-cholines, acetyl-salicylic acid, methylphenydate, procaine, heroin, cocaine,

The most common biotransformation pathway of the ester group is hydrolysis. Hydrolysis of drugs results in most cases in loss of biological activity, whereas the contrary happens to prodrugs. The quantitation of ester hydrolysis is therefore an important element to reveal details of drug metabolism and prodrug design. The hydrolysis rate is well-known to depend on extramolecular factors such as temperature, solvent, pH. Much fewer, and largely qualitative data are available only on how the state of protonation and conformation of the intramolecular vicinity influence the parameters of ester hydrolysis.

The two best-known, infamous “hard drug” agents, heroin and cocaine are molecules that uniformly bear two

ester and one basic amine moieties. Their partial hydrolysis yields intermediate products of one ester and two acid-base functions, the states of which heavily influence their effects. The exact kinetic and thermodynamic description of these systems needs new methods.

Methylphenidate, the active agent of Ritalin, is a central nervous system (CNS) stimulant, it is used in the treatment of attention deficit hyperactivity disorder (ADHD) and narcolepsy. The molecule contains one ester group and it can exist in two protonation states. Its species-specific hydrolysis is described with two hydrolysis rate constants.

Figure 1 summarizes the structures of the studied molecules

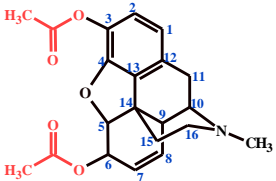
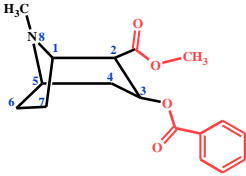
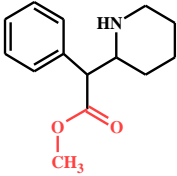
		
3,6-diacetylmorphine	cocaine	methylphenidate

Figure 1. Constitutional formulas of the studied structures.

2. Objectives

The aim of this work was to investigate site- and species-specific hydrolysis rates of cocaine, heroin and methylphenidate.

In kinetic terms, the hydrolysis of cocaine and heroin is a highly complex process. Since hydrolyses take place, at two sites – site 2 and 3 in the case of cocaine; site 3 and 6 in the case of heroin -, and each of the first-

step decomposition products further hydrolyzes, simultaneous and consecutive hydrolyses coexist, thus the hydrolysis rates of the intermediate products had to be determined in order to calculate the site- and species-specific hydrolyses rates of the above-mentioned molecules (cocaine, heroin)

Our aim was also to determine the macroscopic and microscopic protonation constants of all the related substances at 310 ± 0.1 K (body temperature).

3. Methods

All experiments were performed at 310 ± 0.1 K (body temperature) and the ionic strength was held constant at 0.15 M using NaCl as auxiliary electrolyte.

Protonation equilibria: Protonation constants were determined by NMR-pH titrations. pH was measured with Metrohm 780 pH meter equipped with a Metrohm 6.0234.110 pH combination electrode. Concentrations of 5 mM were used in all titration experiments. All pH data are pH meter readings based upon NBS primary standards: 0.05M potassium tetroxalate (pH=1.690), 0.05M potassium hydrogen phthalate (pH=4.022), 0.025M KH_2PO_4 + 0.025M Na_2HPO_4 (pH=6.841) and 0.01M borax buffer (pH=9.088). The substances were dissolved in 0.01M HCl and in 0.01M NaOH solutions containing 5% (v/v) deuterium oxide. The solutions were mixed, pH was measured, and the ^1H NMR spectra were recorded. Evaluation of the titration data was performed with Origin 8 for Windows.

Kinetic studies: The progress of decomposition was monitored and quantified by NMR experiments. Processes were followed in situ: the decomposition took place in the NMR tube. Hydrolysis experiments were carried out in buffered solutions containing 0.05 M NaH_2PO_4 and 0.025 M $\text{Na}_2\text{B}_4\text{O}_7$ at different pH values. 2-5 mg substance of the morphine-ester in question was separately dissolved in 2.00 mL buffer solutions containing 5% D_2O . Its pH was set using NaOH or H_3PO_4 solutions. The progress of hydrolysis was followed gradually by NMR spectroscopy until at least 75 % decomposition. NMR spectra were recorded at $\text{pH} < 8.5$ in every 20-30 minutes, and in every 5-7 minutes at $\text{pH} \geq 8.5$. Pyrazine was used as an internal standard for the integration. Integrals of the aromatic protons, N-methyl protons and methoxy protons are the best linear measures of concentration, since the applied water suppression disturbs the shape of the proton peaks in the vicinity of the solvent signal. Evaluation of the kinetic data was performed with Origin 8 for Windows.

4. Results

The species-specific hydrolysis of methylphenidate is described with two hydrolysis rate constants. The protonated form hydrolyses 80 times faster than the non-charged form.

The hydroxide-catalyzed non-enzymatic, simultaneous and consecutive hydrolyses of diacetylmorphine (DAM, heroin) and cocaine are quantified in terms of 10 site- and species-specific rate constants in connection with also 10 site- and species-specific acid-base equilibrium constants, comprising all the 12 coexisting species in solution. The characterization of the species- and site-specific hydrolysis rate constants involves the major and minor decomposition pathways: via 6-acetylmorphine and 3-acetylmorphine, respectively, and morphine, the final product in the case of heroin; benzoylecgonine and ecgonine methyl ester, respectively and ecgonine in the case of cocaine.

We determined that, the hydrolysis of morphine esters is 18 -120 times faster at site 3 than at site 6, depending on the status of the amino group and the rest of the molecule.

Ecgonine esters have been found to hydrolyze 10 - 250 times faster at site 2 than at site 3, depending on the status of the amino site and the rest of the molecule.

Hydrolysis rate constants are interpreted in terms of intramolecular inductive effects and the concomitant local electron densities. Hydrolysis fraction, a new physico-chemical parameter is introduced and determined to quantify the contribution of the individual microspecies to the overall hydrolysis.

5. Conclusions

In my PhD thesis three ester type molecules were investigated in the terms of protonation- and hydrolysis rate constants.

We determined either site- and species-specific complex ester-hydrolysis rates of heroin, cocaine, methylphenidate and its hydrolysis products at 37 °C using NMR spectroscopy, and a new custom-tailored evaluation method.

In theoretical aspect, this work is the first attempt to handle and decompose overlapping simultaneous and consecutive kinetic processes into quantified hydrolysis rates of every single protonation stage, revealing intramolecular effects that influence electron density at the ester group and the concomitant hydrolysis rates.

Our data also provide information on the hydrogen ion binding propensities of the basic sites in the molecules studied and the related pharmacokinetic and pharmacodynamic behavior, in which also hydrogen

bondings tether the molecules to the active loci of the decomposing enzymes.

This in-depth analysis is also a submolecular map with synthetic drug development perspectives by side-chain amendments to increase or decrease covalent stability and binding capacity for molecules from e.g. the opiate family.

6. Publications

Papers of the thesis work

1. **Levente Szöcs**, Gábor Orgován, Gergő Tóth, Márta Kraszni, Lajos Gergó, Sándor Hosztafi, Béla Noszál. (2016) *Site-and* Species-specific Hydrolysis Rates of Heroin, Eur J Pharm Sci, **89**:105-114,
2. **Levente Szöcs**, Ákos Urai, Gergely Völgyi, Gergő Tóth, Sándor Hosztafi, Béla Noszál (2017) *Site-and* Species-specific Hydrolysis Rates of Cocaine J Pharm Biomed Anal, 2017, **145**:372-378

Related papers

1. Rusu Aura, Gergo Toth, **Levente Szöcs**, József Kökösi, Márta Kraszni, Árpád Gyéresi, Béla Noszál. (2012) Triprotic Site-specific Acid-base Equilibria and Related Properties of Fluoroquinolone Antibacterials

J Pharm Biomed Anal, **66**:50-57

2. Zoltán-István Szabó, **Levente Szöcs**, Daniela-Lucia Muntean, Béla Noszál, Gergő Tóth. (2016) Chiral Separation of Uncharged Pomalidomide Enantiomers Using Carboxymethyl-beta-cyclodextrin: A Validated Capillary Electrophoretic Method

Chirality, **28** (3):199-203

3. Zoltán-István Szabó, Foroughbakhshfasaei Mohammadhassan, **Levente Szöcs**, József Nagy, Balázs Komjáti, Béla Noszál, Gergő Tóth. (2016) Stereoselective Interactions and Liquid Chromatographic Enantioseparation of Thalidomide on Cyclodextrin-bonded Stationary Phases

J Incl Phenom Macrocycl Chem, 85 (3):227-236

4. Zoltán-István Szabó, **Levente Szöcs**, Balázs Komjáti, József Nagy, Béla Noszál, Gergő Tóth. (2016) LC-MS Enantioseparation of Pomalidomide on Cyclodextrin-bonded Chiral Stationary Phases and Elucidation of Chiral Recognition Mechanisms by NMR Spectroscopy and Molecular Modeling

J Sep Sci, **39** (15):2941-2949

5. Ákos Urai, András Váradi, **Levente Szöcs**, Balázs Komjáti, Valerie Le Rouzic, Amanda Hunkele, Gavril Pasternak, Susruta Majumdar, Sándor Hosztafi. (2017) Synthesis and Pharmacological Evaluation of Novel Selective MOR Agonist 6 β -Pyridinyl Amidomorphines Exhibiting Long-lasting Antinociception

Med Chem Comm, **8**:152-157

6. Zoltán-István Szabó, Réka Gál, **Levente Szöcs**, Róbert Ludmerczki, Daniela-Lucia Muntean, Béla Noszál, Gergő Tóth:

Validated capillary electrophoretic method for the enantiomeric quality control of R-praziquantel

Electrophoresis, 2017, in press