Abstract

The betaine/GABA transporter 1 (BGT1) is one of the four GABA transporter (GAT) subtypes that have been cloned from the mammalian brain. While its cerebral expression is well documented, its physiological function and therapeutic potential are poorly understood and subject to controversy. This is partly a result of the lack of pharmacological tools of sufficient potency and subtype-selectivity. Thus, the aim of the present project was to identify and pharmacologically characterize new tool compounds that can aid in assessing the role and relevance of BGT1 in physiological and pathophysiological conditions.

Using radioligand-based uptake experiments at GATs recombinantly expressed in mammalian cell lines, we identified ATPCA (2-amino-1,4,5,6-tetrahydropyrimidine-5-carboxylic acid) as the first BGT1-selective substrate-inhibitor with high potency (IC_{50} 2.5 µM). The structure-activity relationship (SAR) of a series of ATPCA analogues showed that only few modifications are allowed in the core structure of ATPCA, inferring limited space in the binding pocket that harbours the molecular interactions with the compound. Computational induced-fit docking studies and molecular dynamics simulations suggested the non-conserved Q299 and E52 in the orthosteric site of the human BGT1 (hBGT1) as molecular determinants for the BGT1-selectivity of ATPCA and its analogues. The validity of this hypothesis and the importance of these residues were subsequently confirmed by a series of site-directed mutagenesis experiments.

ATPCA was converted into a tritiated analogue, [^{3}H]ATPCA, which was evaluated for its utility to study the function of BGT1 in recombinant and native preparations. Among the four GATs, [^{3}H]ATPCA was shown to be a BGT1-selective substrate with a K_m of 21 µM. Sodium-dependent [^{3}H]ATPCA uptake was detected in cultured cortical mouse neurons, but not astrocytes.

The previously reported BGT1 inhibitor with high nanomolar potency (IC_{50} 590 nM), TK4 ((1*S*,2*S*,5*R*)-5-aminobicyclo[3.1.0]hexane-2-carboxylic acid) was further examined in this project and shown to be a competitive inhibitor of BGT1. *In silico*-guided mutagenesis experiments revealed that Q299 and E52 govern the BGT1-selective profile of also this compound.

Finally, the SAR of a series of novel non-competitive BGT1 inhibitors was evaluated, and the experimental and computational attempts to delineate the binding site are described.
Collectively, this PhD project provides an extensive pharmacological evaluation of new and known BGT1-selective competitive, non-competitive, and substrate-inhibitors that could prove to be instrumental for the further examination of the physiological and therapeutic role of BGT1.