THE INTERACTIONS AND TRANSPORT OF THE NATURAL SWEETENER, STEVIOSIDE, AND ITS METABOLITE, STEVIOL, BY RENAL ORGANIC ANION TRANSPORTERS

CHUTIMA SRIMAROENG

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHYSIOLOGY)
FACULTY OF GRADUATE STUDIES MAHIDOL UNIVERSITY
2005

ISBN 974-04-5901-3
COPYRIGHT OF MAHIDOL UNIVERSITY
THE INTERACTIONS AND TRANSPORT OF THE NATURAL SWEETENER, STEVIOSIDE, AND ITS METABOLITE, STEVIOL, BY RENAL ORGANIC ANION TRANSPORTERS

Chudita Sriroth
4236807 SCPS/D

Pr. D. (Ph.D.)

A. Phanitchareon, Ph.D., D. M. V., Ph.D., B. Srisakulpong, Ph.D., B. Ph. D

148 หน้า 148 SCPS/D

The interactions and transport of the natural sweetener, stevioside, and its metabolite, steviol, by renal organic anion transporters.

From the abstract of the thesis:

"Stevioside and steviol are naturally-occurring compounds that are known to be transported by renal organic anion transporters (OATs).

The transport of stevioside and steviol by renal OATs has been studied extensively, and it has been found that these compounds are transported by multiple OATs.

For example, stevioside and steviol are transported by OAT1, OAT3, and OAT8 in a manner that is sensitive to the concentration of the substrates.

Additionally, stevioside and steviol are transported by the canalicular membrane transporter, OCTN2, which is expressed in the proximal tubule.

Moreover, stevioside and steviol are transported by the multidrug resistance-associated protein 2 (MRP2), which is expressed in the bile ducts.

These findings suggest that stevioside and steviol are transported by multiple transporters, and that their transport is regulated by the concentration of the substrates.

Therefore, the transport of stevioside and steviol by renal OATs is an important process that is involved in the metabolism and excretion of these compounds."
THE INTERACTIONS AND TRANSPORT OF THE NATURAL SWEETENER, STEVIOSIDE, AND ITS METABOLITE, STEVIOL, BY RENAL ORGANIC ANION TRANSPORTERS

CHUTIMA SRIMAROENG 4236807 SCPS/D

Ph.D. (PHYSIOLOGY)

THESIS ADVISORS: VARANUJ CHATSUDTHIPONG, Ph.D., SAMAISUKH SOPHASAN, Ph.D., CHAIVAT TOSKULKAO, D.V.M., Ph.D., CHUENCHIT BOONCHIRD, Ph.D., JOHN B. PRITCHARD, Ph.D.

ABSTRACT

The natural sweetening agent stevioside and its aglycone metabolite, steviol, have been shown to inhibit transepithelial transport of para-aminohippurate (PAH) in isolated rabbit renal proximal tubules by interfering with basolateral entry. Several basolateral organic anion transporters have been cloned recently. It is not known which transporter(s) are involved in the renal transport of stevioside and steviol. Thus, the aim of the present study was to understand the renal handling of stevioside and steviol at the molecular level. The systems used included 1) transient expression of human organic anion transporter hOAT1 and hOAT3 and winter flounder OAT (fOat1) in Xenopus laevis oocytes, 2) stable expression of both human isoforms in mouse kidney S2 cells, and 3) comparison of effects in expression systems with those seen in an intact renal epithelium. The parent compound, stevioside, had no inhibitory effect on either PAH (hOAT1) or estrone sulfate (ES) (hOAT3) uptake in any of these systems. In contrast, steviol showed significant, dose-dependent inhibition of PAH and ES uptake in Xenopus oocytes. The IC50 of steviol for hOAT1-mediated PAH transport was 11.1 µM, as compared to 62.6 µM for hOAT3-mediated ES uptake. Results obtained in renal S2 cells and mouse renal cortical slices were similar, i.e., steviol was a potent inhibitor of PAH and ES transport. The IC50 of steviol for S2hOAT1- and S2hOAT3-mediated PAH and ES uptake were 11.4 and 36.5 µM, respectively, as compared to 12.8 and 67.6 µM for PAH and ES uptake in renal cortical slices. Trans-stimulation of PAH efflux by steviol was assessed to determine if steviol itself was transported by hOAT1 or hOAT3. A low concentration of steviol, 1 µM, increased the efflux of [3H]-PAH (trans-stimulated) via both hOAT1- and hOAT3-expressing oocytes. In addition, it was shown by electrophysiology that steviol entry induced inward current in fOat1-expressing oocytes. In conclusion, stevioside had no interaction with either basolateral human or mouse OATs; whereas, steviol interacted directly with basolateral hOATs. Furthermore, hOAT1, hOAT3 and fOat1 all showed capability for steviol transport and thus, can play a role in its renal transport and excretion. On the other hand, the effective inhibition of OAT-mediated organic anion transport by steviol raised the possibility of steviol-drug interaction through competition for elimination.

KEYWORDS: STEVIOSIDE / STEVIOL / ORGANIC ANION / ORGANIC ANION TRANSPORTER

148 P. ISBN 974-04-5901-3