Research Article

Calmodulin-associated post-translational regulation of rat organic cation transporter 2 in the kidney is gender dependent


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Abstract. In this work, regulation of organic cation transporter type 2 from rat (rOCT2) stably transfected in HEK293 cells was investigated by microfluorimetry with 4-(4-(dimethylamino)styryl)-N-methylpyridinium as substrate. The transport mediated by rOCT2 was specifically stimulated by PKA, phosphatidylinositol-3-kinase, p56
^1^lk tyrosine kinase, mitogen-extracellular-signal-regulated-kinase-1/2, calmodulin (CaM), and CaM-kinase-II. The regulatory pattern of rOCT2 differs markedly quantitatively and qualitatively from that of other OCT isoforms. Only CaM-dependent upregulation is conserved throughout the OCT family. For this reason, CaM regulation of rOCT2 was also investigated in isolated S3-segments (known to express only rOCT2) of male and female rat proximal tubules. Inhibition of CaM by calmidazolium significantly decreased rOCT2 activity (-49.0 ± 13.6%, n = 4) in male but not female (9.0 ± 13.0%, n = 4) rats. Real-time PCR and Western blot investigations of CaM expression in rat kidneys showed that male animals have significantly higher CaM expression. This is the first study describing post-translational gender-dependent rOCT2 regulation.

Keywords. Organic cation transporter, physiology, regulation, kidney, gender dependence.

Introduction

Organic cation transporters (OCTs) are membrane proteins that have important physiological, pharmacological, and toxicological implications because of their role in the transport of endogenous organic cations, such as the dopaminergic neuromodulators histidyl-proline diketopiperazine and salsolinol [1], histamine [2] and xenobiotica such as metformin [3], platin-derivatives [4–6] and paraquat [7]. The transport mediated by OCTs has been characterized as polyppecific, bidirectional and electrogenic [8]. Three OCT-isoforms (OCT1, OCT2 and OCT3), mainly expressed in epithelia of intestine, liver, brain and kidney in a species- and isoform-specific fashion, have been identified in rat, mouse and man [9]. Common structural properties of OCTs are the presence of 12 putative α-helical transmembrane domains (TMD) with a large hydrophilic extracellular loop between TMD1 and TMD2 and a big intracellular loop between TMD6 and TMD7 containing several potential protein kinase phosphorylation sites [10].

The rat OCT2 (rOCT2, also called solute carrier 22a2) is mainly expressed in the kidney at the basolateral membrane of S2 and S3 segments of the proximal...
tubule in the outer stripe of the outer medulla [11, 12].
Rat OCT1 is also highly expressed in the kidney, but it is
localized at the basolateral membrane of S1 and S2
segments of superficial and juxtamedullary proximal
convoluted tubules [13]. The human kidney expresses
mainly the hOCT2 isoform on the basolateral cell
membrane of the entire proximal tubule [14].
Compared to female rats, males have a higher
expression of rOCT2 both at mRNA and protein
levels, which is dependent on testosterone [15, 16].
Androgen response elements (AREs) in the 5'-flanking
region of the rOCT2 gene are responsible for
stimulation of rOCT2 promoter activity by testoster-
one [17].
Although regulation of different OCT isoforms has
been extensively studied [18–22], there is no infor-
mation available about regulation of rOCT2. Since the
rat is a commonly used animal model for physiolog-
ical, pharmacological and toxicological studies, in this
work the properties of rOCT2 expressed in HEK-293
cells, with special emphasis on its regulation by
different intracellular pathways, were investigated.
Furthermore, the gender-dependency of regulation
was also examined in freshly isolated rat proximal
tubular S3 segments from male and female rats.

Materials and methods

HEK293 Cell culture. Experiments were performed
with human embryonic kidney (HEK)-293 cells
(CRL-1573; American Type Culture Collection,
Rockville, MD), which stably express rOCT2 or
rOCT1. rOCT2 or rOCT1 was cloned and stable
transfection was performed as described earlier [23,
24]. Cells were grown at 37 °C in 50 ml cell culture
flasks (Greiner, Frickenhausen, Germany) in DMEM
(Biochrom, Berlin, Germany) containing 3.7 g/l
NaHCO\textsubscript{3}, 1.0 g/l D-glucose, and 2.0 mM L-glutamine
(Biochrom), and gassed with 8% CO\textsubscript{2}.

Method 1. The fluorescence measurement device and
experimental procedures used in this method were
already described in detail [18, 20, 25]. Measurements
were performed in the dark with an inverted micro-
scope (Axiovert 135; Zeiss, Oberkochen, Germany)
equipped with a 100x oil immersion objective.
Excitation light (450 to 490 nm) was reflected by a
dichroic mirror (560 nm) to a perfusion chamber. Cell
monolayers on cover slips formed the bottom of the
chamber. The preparations were superfused at a rate
of 10 ml/min with a HCO\textsubscript{3}\textsuperscript{-} free Ringer-like solution
containing (in mmol): NaCl 145, K\textsubscript{2}HPO\textsubscript{4} 1.6, KH\textsubscript{2}PO\textsubscript{4} 0.4,
D-glucose 5, MgCl\textsubscript{2} 1, calcium gluconate 1.3,
and pH adjusted to 7.4 at 37 °C. Fluorescence emission
(575 to 640 nm) was measured by a photon counting
tube (Hamamatsu H 3460–04; Herrsching, Ger-
many). To directly determine the affinity of rOCT2 for
ASP\textsuperscript{+}, saturation experiments at 37 °C and 8 °C were
performed as already described [26]. This approach
was also used to determine K\textsubscript{m} and V\textsubscript{max} under the
regulatory influence of calmodulin inhibition. To
study regulation of rOCT2, ASP\textsuperscript{+}-uptake was evalu-
at after incubation with the respective agonists or
inhibitors in the continued presence of these substan-
ces and compared to that observed in control experi-
ments performed under the same conditions without
agonists or inhibitors. Results are expressed as
changes of ASP\textsuperscript{+}-uptake in percentage of control
experiments.

Isolation of tubular segments for microfluorimetry.
Lewis–Brown–Norway (LBN) rats (240–290 g,
Charles River, Sulzfeld, Germany) with free access
to standard rat chow (Ssniff, Soest, Germany) and tap
water were used. Experiments were approved by a
governmental committee on animal welfare and were
performed in accordance with national animal pro-
tection guidelines. Proximal tubules (S3 segments)
were mechanically isolated in MEM-EARLE medi-
un (Biochrom), transferred to a perfusion chamber,
and fixed by two holding pipettes closing the lumina at
the ends of the tubule segment for microfluorimetric
measurements. In this way, only the basolateral side of
the tubules, where rOCT2 is located [12], could be
reached by the experimental solutions.

Fluorescence measurements. As substrate for OCTs,
the fluorescent organic cation 4-(4-(dimethylamino)s-
teryl)-N-methylpyridinium (ASP\textsuperscript{+}) at a concentra-
tion of 1 μM was used. The ASP\textsuperscript{+}-uptake by rOCT2
expressing cells was measured by two methods:

Method 1. The fluorescence measurement device and
experimental procedures used in this method were
already described in detail [18, 20, 25]. Measurements
were performed in the dark with an inverted micro-
scope (Axiovert 135; Zeiss, Oberkochen, Germany)
equipped with a 100x oil immersion objective. Ex-
citation light (450 to 490 nm) was reflected by a
dichroic mirror (560 nm) to a perfusion chamber. Cell
monolayers on cover slips formed the bottom of the
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of 10 ml/min with a HCO\textsubscript{3}\textsuperscript{-} free Ringer-like solution
containing (in mmol): NaCl 145, K\textsubscript{2}HPO\textsubscript{4} 1.6, KH\textsubscript{2}PO\textsubscript{4} 0.4,
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