Progress in Neuro-Psychopharmacology & Biological Psychiatry 49 (2014) 1–6

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

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

Inhibition of organic cation transporter 2 and 3 may be involved in the mechanism of the antidepressant-like action of berberine

Siyuan Sun [1], Kai Wang [1], Hongmei Lei, Liping Li, Meijuan Tu, Su Zeng, Hui Zhou *, Huidi Jiang *

Department of Pharmaceutical Analysis and Drug Metabolism, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, Zhejiang, PR China

ARTICLE INFO

Article history:
Received 27 September 2013
Received in revised form 8 November 2013
Accepted 8 November 2013
Available online 15 November 2013

Keywords:
Antidepressant
Berberine
Organic cation transporter 2
Organic cation transporter 3
Uptake-2

ABSTRACT

Organic cation transporter 2 (OCT2) and 3 (OCT3) are low-affinity, high-capacity transporters (uptake-2) expressed in the central nervous system (CNS) and other major organs. Proven to be essential components in the CNS functions, OCT2 and OCT3 are suggested as potential targets of antidepressant therapeutics recently. Berberine, an active constituent derived from many medicinal plants, such as *Coptis chinensis*, has been reported to possess antidepressant-like action in the tail suspension test and forced swim test with elevated serotonin/norepinephrine/dopamine (5-HT/NE/DA) level in mouse brain; however the mechanism has not been elucidated. In consideration of the relation between OCT2/3 and antidepressant action, and the characteristic of berberine as an organic cation, we investigated the potential involvement of OCT2 and OCT3 in the antidepressant-like action of berberine in the present study. The results in mouse brain synaptosomes demonstrated that uptake-2 inhibition might play a notable role in enhanced serotonergic and noradrenergic effects induced by berberine. The inhibitory study in transfected MDCK cells displayed that berberine is a potent inhibitor of human OCT2 and OCT3, and its IC_{50} values for inhibition of transporter-mediated 5-HT/NE uptake are between 0.1 and 1 μM. In addition, berberine was identified as a substrate of hOCT2 and hOCT3. In conclusion, berberine is a substrate and an inhibitor of hOCT2 and hOCT3, and its inhibition on OCT2- and OCT3-mediated 5-HT and NE uptake may contribute to the enhanced monoamine neurotransmission in mouse brain. It was deduced that the inhibition of OCT2 and OCT3 probably be implicated in the mechanism of antidepressant-like action.

1. Introduction

Depression is one of the top ten causes of morbidity and mortality worldwide based on a survey by the World Health Organization (Berton and Nestler, 2006). Due to the complexity of depression, its precise pathophysiological basis remains obscure despite decades of wide and continuous research. The extensively adopted monoamine hypothesis of depression proposes that the underlying biological or neuroanatomical basis for depression is a deficiency of central noradrenergic and/or serotonergic systems. Targeting this neuronal lesion with an antidepressant would tend to restore normal function in depressed patients (Hirschfeld, 2000). All available antidepressant medications, which are based on serendipitous discoveries of the clinical efficacy of two classes of antidepressants (tricyclic and monoamine oxidase inhibitor antidepressants) more than 50 years ago (Berton and Nestler, 2006), act via the monoamine neurotransmitters, serotonin (5-HT) or norepinephrine (NE). Two distinct transport systems, named uptake-1 and uptake-2, are responsible for the clearance of monoamine neurotransmitters released into the synaptic cleft (Eisenhofer, 2001). Uptake-1 consists of Na^+- and Cl^--dependent, high-affinity, and low-capacity transporters in the solute carrier (SLC) 6 family and includes 5-HT transporter (SERT, SLC6A4), dopamine transporter (DAT, SLC6A3), and norepinephrine transporter (NET, SLC6A2). Primarily expressed on the nerve endings of monoaminergic neurons, uptake-1 transporters are the major pathway for removing released transmitters from the extracellular space, and the targets for numerous antidepressants (Torres et al., 2003). Uptake-2 is composed of Na^+- and Cl^--independent, low-affinity, and high-capacity transporters. Once largely overlooked before, the contribution of uptake-2 in monoamine neurotransmissions is considered to be indispensable, and receives increasing attention from researchers. Organic cation transporters (OCTs) and plasma membrane monoamine transporter (PMAT) are among the most efficient uptake-2 transporters for 5-HT, NE, and other biogenic amine neurotransmitters (Daws et al., 2013).

The polyspecific OCT2 (SLC22A2) and OCT3 (SLC22A3), belonging to the SLC22 family, translocate organic cations and weak bases, encompassing cationic drugs, toxins and environmental waste products, in an electrogenic manner (Koepsell et al., 2007). Compared to OCT1 and OCT2, OCT3 is more wildly and densely distributed in many brain regions, particularly rich in cerebellum, subfornical organ, dorsal

Abbreviations: hOCT, human organic cation transporter; MDCK, Madin-Daby canine kidney; 5-HT, serotonin; NE, norepinephrine; MPP^+, 1-methyl-4- phenylpyridinium; LC-MS/MS, liquid chromatography-tandem mass spectrometry.

* Corresponding authors at: Department of Pharmaceutical Analysis and Drug Metabolism, College of Pharmaceutical Sciences, Zhejiang University, 866 Yuhangtang Road, Hangzhou 310058, PR China. Tel./fax: +86 571 88208408.
E-mail addresses: zhouhui@zju.edu.cn (H. Zhou), hdjiang@zju.edu.cn (H. Jiang).

[1] Contributed equally to this study.

0278-5846/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.pnpbp.2013.11.005

raphe, hypothalamic nuclei, cortex, and hippocampus (Amphoux et al., 2006; Koepsell et al., 2007). OCT2 is enriched in the limbic system, especially in frontal cortex, cornu ammonis 1 and 3 of hippocampus, and amygdala (Bacq et al., 2012). It was revealed in recent studies that OCT3 is an important complementary transporter in central nervous system (CNS) function as well as neuropsychiatric disorders, and OCT2 is implicated in anxiety and depression-related behaviors (Baganz et al., 2010; Cui et al., 2009; Gasser et al., 2006; Rappold et al., 2011; Wultsch et al., 2009; Zhu et al., 2010). Moreover, the OCT inhibitors produce or potentiate antidepressant-like effects in rodent models when uptake 1 transporters were impaired or antagonized (Bacq et al., 2012; Baganz et al., 2010; Horton et al., 2013; Mooney et al., 2008; Rahman et al., 2008). All the evidence suggested OCT2 and OCT3 as novel targets in antidepressant therapeutics.

Berberine is a quaternary isoquinoline alkaloid derived from various medicinal plants including *Coptis chinensis* (coptis or goldenthread), *Berberis vulgaris* (barberry), *Hydrastis canadensis* (goldenseal), and *Mahonia aquifolium* (Oregon grape). It has been reported that berberine possesses various pharmacological activities, such as antidiabetic (Lee et al., 2006), anti-inflammatory (Ivanovska and Philipov, 1996), antimicrobial (Schmeller et al., 1997), anti-amnesic (Peng et al., 1997), anti-malarial (Park et al., 2003), and anxiolytic-like effects (Peng et al., 2004). Clinically, berberine is used to treat diarrhea, and is an effective and safe antisecretory drug in the context (Rabbani et al., 1987). Recent reports demonstrated that berberine could produce antidepressant-like action in the forced swim test and in the tail suspension test in mice with elevated 5-HT/NE/DA levels in the brains. Some pathways were proposed, whereas the precise mechanism is still elusive (Kulkarni and Dhir, 2007, 2008; Peng et al., 2007).

Since the chemical structure of berberine fits in well with substrate characteristics of OCTs, it is highly possible that berberine will interact with OCTs. In view of the importance of OCT2 and OCT3 in antidepressant therapeutics, we investigated the interaction between berberine and OCT2/OCT3 in the present study. The results may help to further explore the antidepressant-like effect of berberine from a novel perspective.

2. Materials and methods

2.1. Materials

Berberine hydrochloride and serotonin hydrochloride were attained from Aladdin Co., Ltd. (Shanghai, China). 1-Methyl-4-phenylpyridinium (MPP^+) iodide, decynium-22, pargyline hydrochloride, and ascorbate were acquired from Sigma-Aldrich (St. Louis, MO). Venlafaxine hydrochloride and norepinephrine bitartrate were purchased from the National Institutes for Food and Drug Control (Beijing, China). Dulbecco's modified Eagle's medium (DMEM), penicillin, streptomycin, and fetal bovine serum (FBS) were obtained from Invitrogen (Carlsbad, CA). Sodium dodecyl sulfonate (SDS) was gained from Amresco (Solon, OH). All of the chemicals or solvents used for UPLC-MS/MS were commercially available and were of HPLC grade.

2.2. Animals

Male ICR albino mice (weighing 25–30 g) were provided by the Experimental Animal Center of Zhejiang Academy of Medical Sciences. They were housed at 22 ± 1 °C with free access to food and water, under a 12:12 h light/dark cycle (lights on 08:00 h). The experimental protocol complied with an approved animal use guidelines of Zhejiang University.

2.3. Cell culture

Madin-Darby canine kidney (MDCK) cells were acquired from the Peking Union Medical College (Beijing, China). The hOCT2-transfected and hOCT3-transfected MDCK cells and their mock cells were established in our laboratory. The culture medium was DMEM containing 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. All the cells were cultured in a 37 °C humidified incubator with 5% CO_2.

2.4. LC-MS/MS analysis

The concentrations of 5-HT, NE, MPP^+, and berberine in samples were determined by a Waters Acquity triple quadrupole mass spectrometer equipped with a Waters Acquity ultra-performance liquid chromatography system. All the compounds were eluted on an Atlantis Silica HILIC Column (3 μm, 2.1 × 50 mm; Waters, Milford, MA) except MPP^+. The mobile phase consisted of solvent A (20 mM ammonium acetate containing 0.1% formic acid) and solvent B (acetonitrile) at a flow rate of 0.3 ml/min flushed in the following gradient: 0–0.5 min (90%–90% B), 0.5–1.0 min (90%–60% B), 1.0–2.0 min (60%–60% B), 2.0–2.1 min (60%–90% B), and 2.1–3.0 min (90%–90% B). The injection volume was 7 μl. Nitrogen was used as the desolvation gas and cone gas, and argon as the collision gas. All the compounds were analyzed in positive electrospray ionization mode and multiple reaction monitoring. The parameters of MS spectrum for detected compounds were listed in Table 1. Data were processed using MassLynx version 4.1 software. The method of MPP^+ analysis had been described in our previous report (Tu et al., 2013).

2.5. Synaptosome preparation and uptake assays

Mouse brain synaptosomes were prepared according to previous report with little modifications (Duan and Wang, 2010). Briefly, mouse was decapitated and the whole brain was quickly removed and homogenized on ice in a homogenizer with ice-cold sucrose homogenization buffer (320 mM sucrose, 5 mM HEPES, pH 7.4). The homogenate was centrifuged at 1000 *g* for 10 min at 4 °C, and the supernatant was centrifuged again at 16,000 *g* for 20 min at 4 °C. The synaptosomal pellet was washed with HBSS and resuspended with uptake buffer (HBSS supplemented with 100 μM pargyline and 100 μM ascorbic acid).

The uptake assays were conducted in a 37 °C shaking water bath. Synaptosomes were preincubated for 3 min, then the incubation started with adding monoamine substrates in the presence or absence of various inhibitors (5-HT: 2.5 μM, NE: 10 μM). After designed incubation time (5-HT: 5 min, NE: 10 min), uptake was terminated by centrifuging at 16,000 *g* for 30 s at 4 °C with the supernatant discarded. The synaptosomes was rinsed thrice with ice-cold PBS, followed by solubilized in 100 μl 0.1% sodium dodecyl sulfonate (SDS). Eighty microliters of the lysates was mixed with 160 μl acetonitrile, vortexed for 2 min and centrifuged at 16,000 *g* for 15 min to precipitate proteins. The supernatant was analyzed by LC-MS/MS. The uptake was normalized to the protein contents in the lysates, which were measured by a BCA protein assay kit (Beyotime, Haimen, China).

2.6. Cellular uptake assays

Transfected or mock MDCK cells were seeded into 24-well plates. When the cells reached confluence after grown for ~2 to 3 days, the culture medium was replaced by Hank's balanced salt solution (HBSS)

Table 1
Mass spectrum parameters of detected compounds.

Compounds	m/z (parent/daughter)	Capillary (kV)	Cone (V)	Collision energy
Berberine	336.34/320.11	3.9	49	28
5-HT	176.88/160.09	4.5	17	12
NE	169.98/151.91	3.1	11	8

Note: m/z (parent/daughter) represents the mass-to-charge ratio of parent ion/daughter ion.