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Hyperammonemia induces transport of taurine and creatine and suppresses claudin-12 gene expression in brain capillary endothelial cells *in vitro*

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Abstract

Ammonia is a key neurotoxin involved in the neurological complications of acute liver failure. The present study was undertaken to study the effects of exposure to pathophysiologically relevant concentrations of ammonium chloride on cultured brain capillary endothelial cells in order to identify mechanisms by which ammonia may alter blood–brain barrier function. Conditionally immortalized mouse brain capillary endothelial cells (TM-BBB) were used as an *in vitro* model of the blood–brain barrier. Gene expression of a series of blood–brain barrier transporters and tight junction proteins was assessed by quantitative real time PCR analysis. Exposure to ammonia (5 mM for 72 h) resulted in significant increases in mRNA levels of taurine transporter (TAUT; 2.0-fold increase) as well as creatine transporter (CRT; 1.9-fold increase) whereas claudin-12 mRNA expression was significantly reduced to 67.7% of control levels. Furthermore, [^{3}H]taurine and [^{14}C]creatine uptake were concomitantly increased following exposure to ammonia, suggesting that up-regulation of both TAUT and CRT under hyperammonemic conditions results in an increased function of these two transporters in TM-BBB cells. TAUT and CRT are respectively involved in osmoregulation and energy buffering in the brain, two systems that are thought to be affected in acute liver failure. Furthermore, claudin-12 down-regulation suggests that hyperammonemia may also affect tight junction integrity. Our results provide evidence that ammonia can alter brain capillary endothelial cell gene expression and transporter function. These findings may be relevant to pathological situations involving hyperammonemia, such as liver disease.

Keywords: Ammonia; Blood-brain barrier; Brain capillary endothelial cells; Claudin-12; Creatine transporter; Taurine transporter

Hyperammonemia is a key factor involved in the pathogenesis of hepatic encephalopathy (HE) and brain edema, two serious neurological complications of acute liver failure (ALF). Under normal physiological conditions, a large amount of ammonia coming from the gut enters the portal circulation and is detoxified in the liver. Therefore, when the liver fails, ammonia rapidly accumulates in the circulation and, as a result of the equilibrium between its ionic and gaseous forms, freely crosses the blood-brain barrier (BBB) and accumulates in the brain (Felipo and Butterworth, 2002). Ammonia affects several important central nervous system (CNS) functions including energy metabolism (Rao and Norenberg, 2001), hemodynamics (Vaquero et al., 2004) and neurotransmission (Felipo and Butterworth, 2002).

Tight junctions formed by brain capillary endothelial cells constitute the blood–brain barrier (BBB) which is essential for

Abbreviations: ABCG2, ATP-binding cassette G2 transporter; ALF, acute liver failure; ASCT2, alanine serine cysteine transporter 2; ATA2, Na^+ dependent small neutral amino acid transporter 2; BBB, blood–brain barrier; CNS, central nervous system; CRT, creatine transporter; GLUT1, glucose transporter 1; LAT1, large neutral amino acid transporter 1; MCT1, monocarboxylate transporter 1; MRP4, multidrug resistance-associated protein 4; TAUT, taurine transporter 1

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isolating the cerebral interstitial fluid from the general circulation. Several transport systems expressed by brain capillary endothelial cells mediate the influx or efflux of specific nutrients and metabolites across the BBB, some of which are thought to play a role under pathological conditions. For instance, it has been reported that BBB transport of cystine into the brain is induced by oxidative stress (Hosoya et al., 2001). This induction could play a protective role in conditions involving oxidative stress, since induction of cystine transport increases the synthesis of glutathione, a major free radical scavenger.

Accordingly, it is likely that events such as oxidative stress (Norenberg et al., 2004) or alterations in brain energy metabolism resulting from hyperammonemia could affect BBB functions and, in turn, alter CNS function. Although no gross BBB breakdown has been observed in ALF (Traber et al., 1987; Kato et al., 1992), some patients with ALF display swelling of brain capillary endothelial cells (Kato et al., 1992) and alterations in BBB permeability have been reported in animal models of ALF and hyperammonemia (Sears et al., 1985; Ziylan et al., 1993; Scorticati et al., 2004). These observations suggest that hyperammonemia might affect the BBB with potential consequences on brain homeostasis.

Acute hyperammonemia causes astrocytic swelling leading to intracranial hypertension which can potentially cause brain herniation and death in severe cases. A growing number of studies have demonstrated that hyperammonemia causes changes in cerebral and, in particular, astrocytic gene expression (see Desjardins et al., 2001 for review). In contrast to the wealth of information available on astrocytes, little is known about the effects of ammonia on the function and gene expression of brain capillary endothelial cells constituting the BBB.

The purpose of the present study was, therefore, to clarify the effects of ammonia on brain capillary endothelial cell gene expression and function in order to identify possible alterations in BBB function in hyperammonemia and ALF. Conditionally immortalized mouse brain capillary endothelial cells (TM-BBB) were used as an *in vitro* model of the BBB since they retain several characteristics of brain capillary endothelial cells, including gene expression and transporter function (Terasaki et al., 2003).

1. Experimental procedures

1.1. Materials

[^{3}H]Taurine (SA: 31.0 Ci/mmol) was purchased from Amersham Biosciences (Piscataway, NJ); [carboxyl-^{14}C] inulin ([^{14}C]inulin; SA: 1.92 mCi/g) was from NEN Life Sciences (Boston, MA); [^{14}C]creatine (SA: 0.053 Ci/mmol) was from American Radiolabeled Chemicals Inc. (St. Louis, MO, USA); taurine was from Wako Pure Chemicals (Osaka, Japan); L-diaminobutyric acid (L-DABA), β-guanidinopropionic acid (β-GP) and NH_4Cl were from Sigma–Aldrich (St. Louis, MO); creatine was from Alfa Aesar (Ward Hill, MA). All other chemicals were of reagent grade and available commercially.

1.2. Cell culture

The TM-BBB4 cell line, a conditionally immortalized mouse brain capillary endothelial cell line established from transgenic mice harboring the temperature-sensitive SV40 large T-antigen gene was used as an *in vitro* BBB model (Hosoya et al., 2000). TM-BBB4 cells were cultured on collagen type-I coated culture dishes (BD Biosciences Bedford, MA, USA) at 33 °C under 5% CO_2 and 95% air as described previously (Hosoya et al., 2000). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Nissui Pharmaceutical Co., Tokyo, Japan) supplemented with 15 μg/mL endothelial cell growth factor (Roche Diagnostics, Indianapolis, IN, USA) and 10% fetal bovine serum (Moregate, Bulimba, Australia). The expanded cells were used between passages 2 and 5.

Table 1
Primer sequences used for RT-PCR

Gene	Sense primer; antisense primer	Product (bp)	Annealing (°C)	Accession
β-Actin	5′-TTTGAGACCTTCAACACCCC-3′; 5′-ATAGCTCTTCTCCAGGGAGG-3′	352	65	NM_007393
ASCT2	5′-CCCCTCCTGAAACAGTACCA-3′; 5′-AGCCTCTCCAGGAAGGAGAC-3′	221	65	NM_009201
ATA2	5′-CTGGTGTCCCTTGTCCTCAT-3′; 5′-AACGTCAGGATGGGTACTGC-3′	296	65	NM_175121
LAT1	5′-CATCATCGGCTCTGGCATCTTCGTG-3′; 5′-TCTGCTGCAGGTTGGACGCATCAC-3′	492	65	AB023409
TAUT	5′-CCTGGGCTTCATGGCACAAG-3′; 5′-GGCAGCGGCATCATGGTTAC-3′	112	65	NM_009320
GLUT1	5′-CTAGAGCTTCGAGCGCAGCGC-3′; 5′-AGGCCAACAGGTTCATCATC-3′	335	60	NM_011400
MCT1	5′-GCCTGAGCAAGTCAAGCTAGAAA-3′; 5′-CATTTGCAACAACAGAAGCAGC-3′	404	60	NM_009196
CRT	5′-TCATGAAGGCCGGCAGCATCAA-3′; 5′-CCCAGGCCAGCACCATGATGTA-3′	120	65	AB077327
Occludin	5′-GTGAGCTGTGATGTGTGTTGAGCT-3′; 5′-GTGGGGAACGTGGCCGATATAATG-3′	402	65	NM_008756
Claudin-12	5′-CGGATGAGGCTAGGAGTTTGTCTG-3′; 5′-CCAGCGCATGAGCACTACCTGAT-3′	417	65	NM_022890
ABCG2	5′-CAATGGGATCATGAAACCTG-3′; 5′-GAGGCTGATGAATGGAGAA-3′	536	60	NM_011920
MRP4	5′-GGTTGGAATTGTGGGCAGAA-3′; 5′-TCGTCCGTGTGCTCATTGAA-3′	222	60	AK157723