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Elevated dopamine induces minimal hepatic encephalopathy by activation of astrocytic NADPH oxidase and astrocytic protein tyrosine nitration

Saidan Ding[a,1], Jianjing Yang[b,1], Leping Liu[a], Yiru Ye[c], Xuebao Wang[d], Jiangnan Hu[b], Bicheng Chen[a], Qichuan Zhuge[b,*]

[a] Zhejiang Provincial Key Laboratory of Aging and Neurological Disease Research, Department of Surgery, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, China
[b] Neurosurgery Department, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, China
[c] Department of Computer, Wen Zhou Medical University, Wenzhou 325000, China
[d] Analytical and Testing Center, Wenzhou Medical University, Wenzhou 325000, China

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ABSTRACT

Background: We previously demonstrated that dopamine (DA) overload may be a key mechanism behind development of minimal hepatic encephalopathy (MHE) in rats. It has been shown that low-grade cerebral oedema and oxidative stress play important roles in the pathogenesis of MHE. In the current study, DA-triggered oxidative injury in cerebral cortex was studied.
Methods: An MHE rat model was used. DA was injected intracerebroventricularly (i.c.v.) into rats and added to primary cortical astrocytes (PCAs). Immunoblotting, immunoprecipitation and immunostaining were conducted after DA injection.
Results: Cognitive impairment and cerebral edema were observed in MHE rats and rats injected with 10 μg DA. Astrocyte swelling was increased by DA. Astrocytic protein tyrosine nitration (PTN) was induced by DA. DA-induced PTN was insensitive to L-NMMA but was blunted by apocynin, superoxide dismutase, catalase and uric acid. Exposure to DA substantially increased levels of astrocytic NADPH oxidase subunits and induced $p47^{phox}$ phosphorylation and reactive oxygen species production but decreased the expression and activity of neuronal-type nitric oxide synthase (nNOS).
Conclusions: PTN induced by DA, which was attributed to NADPH oxidase and not to nNOS, may alter astrocyte function and thereby contribute to the precipitation of MHE episodes.

1. Introduction

Minimal hepatic encephalopathy (MHE) is a neurocognitive disorder that affects up to 80% of cirrhotic patients (Montgomery and Bajaj, 2011). Subtle changes in cognitive function, electrophysiological parameters, cerebral neurochemical/neurotransmitter homeostasis, cerebral blood flow, metabolism, and fluid homeostasis can be observed in cirrhosis patients without hepatic encephalopathy (HE) (Dhiman and Chawla, 2009). Morphological abnormalities of the brain have been identified in this population, such as mild brain edema, hyperintensity of the globus pallidus and other subcortical nuclei observed in cerebral MR studies, and the central and cortical atrophy observed in neural imaging studies; however, these morphological abnormalities are unlikely to have diagnostic utility. Similarly to overt HE, oxidative stress plays key roles in the pathogenesis of MHE (Montgomery and Bajaj, 2011). However, the exact pathogenesis of MHE remains unknown (Torres et al., 2013).

Intracellular glutamine accumulation caused by increased ammonia detoxification leads to astrocyte swelling (Willard-Mack et al., 1996), which is a recognized early pathogenic event in MHE in cirrhotic patients (Häussinger, 2006) and may contribute to the severe rise in intracranial pressure in patients with fulminant hepatic failure (Blei and Larsen, 1999). Under pathophysiological circumstances, the interplay of multiple factors may account for astrocyte swelling. For example, in MHE, with liver disease and portosystemic shunting, inefficiently detoxified gut-derived toxins

* Corresponding author at: The First Affiliated Hospital of Wenzhou Medical University, Shangcai Village, Wenzhou City, Zhejiang Province, China.
Tel.: +86 13676768666.
E-mail address: firstdsdan@hotmail.com (Q. Zhuge).
[1] Saidan Ding and Jianjing Yang contributed equally to this work.

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(eg, ammonia, benzodiazepine-like substances) will accumulate in the blood, cross the blood-brain barrier (BBB), and result in altered neurotransmission and astrocyte swelling (Prakash et al., 2013). It was hypothesized that dopamine (DA), a confirmed MHE-precipitating factor (Ding et al., 2013), contributes at least in part to astrocyte swelling.

Astrocytes are involved in water homeostasis and edema formation (Gill et al., 1973). Astrocyte swelling can increase reactive oxygen species (ROS) and NO production, which cause protein tyrosine nitration (PTN); this is induced, for example, by toxins relevant for HE in quantitites sufficient to produce oxidative stress and PTN and thus contributing to altered astrocytic and neuronal function (Görg et al., 2013; Lachmann et al., 2013). ROS mediate astrocyte swelling induced by glutamate (Bender et al., 1998; Dombro et al., 2000) or ammonia (Norenberg et al., 2005). PTN in astrocytes, the consequence of oxidative/nitrosative stress, is induced by hypoosmotic astrocyte swelling (Schliess et al., 2002; Görg et al., 2003, 2006; Schliess et al., 2004). Therefore, we assumed that astrocyte swelling may account for cerebral PTN caused by DA overload in MHE.

Oxidative/nitrosative stress has a variety of functional consequences, which are considered to be crucial in the pathogenesis of HA. Examples include PTN attributed to ONOO-production (Görg et al., 2013). The respective production of superoxide anion radical (O_2^-) and NO leading to ONOO-synthesis is triggered through activation of NADPH oxidase and neuronal-type nitric oxide synthase (nNOS) (Schliess et al., 2002). NADPH oxidase and nitric oxide synthase (NOS) are major contributors to early ROS and NO formation (Kruczek et al., 2009; Reinehr et al., 2007). NADPH oxidase is composed of a catalytic moiety (gp91), which is activated by assembly with regulatory proteins including $p47^{phox}$, $p67^{phox}$, and Rac (Bokoch and Diebold, 2002; Nauseef, 2004; Pani et al., 2001; Vignais, 2002). Serine phosphorylation of the cytosolic subunit $p47^{phox}$ relieves its inhibitory intramolecular interaction and is critical for $p47^{phox}$-dependent NADPH oxidase activation (Groemping et al., 2003; Johnson et al., 1998; Park and Babior, 1997).

Our previous study found that the pathogenesis of MHE may be associated with elevated DA of cirrhotic livers: excessive DA from livers crosses the BBB and inhibits learning and memory formation (Ding et al., 2013). In the current study, we investigated the effect of DA on activation of NADPH oxidase, NO production, and PTN both in rat brain in vivo and in cultured rat astrocytes.

2. Materials and methods

2.1. MHE models

A total of 50 Sprague–Dawley rats (Experimental Animal Center of The Chinese academy of sciences in shanghai) weighing 220–250 g were used. All animals were subjectred to series of behavioral tests: Y-maze (YM), open-field tests (OF), elevated-plus maze (EPM), and water-finding task (WFT). Rats were then randomly divided into 2 groups: control group ($n = 10$) and thioacetamid (TAA) group ($n = 40$). MHE was induced by intraperitoneal (i.p.) injection of TAA (200 mg/kg in normal saline, Sigma–Aldrich) twice per week for 8 weeks (Jia and Zhang, 2005). After 8 weeks, the behavioral tests were performed for all rats again. Criteria of MHE: (a) values of YM were lower than 3/4-fold average normal values, (b) values of WFT were more than 3/2-fold average value normal values, (c) EEG showed no typical slow wave of hepatic encephalopathy (HE) (Jia and Zhang, 2005). If TAA-treated rats met the criteria of either (a) + (c) or (b) + (c), rats were included in the MHE group. Liver/serum/cerebral cortex were collected for Fluorescent staining, immunoblotting and determination of DA.

2.2. DA-treated rat models

Intracerebroventricular (i.c.v.) injection of dopamine hydrochloride (1 μg/3 μl and 10 μg/3 μl in saline) was stereotaxically performed in the left lateral ventricles of rats (anterior–posterior, +0.3 mm; lateral, 1.0 mm; horizontal, 3.0 mm from the bregma) ($n = 15$). At 7 days after injection, rats were performed for an OF test, a YM, an EPM test and a WFT test.

2.3. Behavioral tests

Open-field tests (OF) were performed as described (Kawasumi et al., 2004). Briefly, rats were individually placed at the center of a 10 × 10 cm gray plastic field (with 20-cm interval black grids) surrounded by a 20-cm wall, and allowed to move freely for 3 min. Ambulation was measured as the total grid line crossing.

The apparatus for Y-maze (YM) was made of gray plastic, with each arm 40 cm long, 12 cm high, 3 cm wide at the bottom and 10 cm wide at the top (Kawasumi et al., 2004; Yamada et al., 2005). The three arms were connected at an angle of 120°. Rats were individually placed at the end of an arm and allowed to explore the maze freely for 8 min. Total arm entries and spontaneous alternation percentage (SA%) were measured. SA% was defined as a ratio of the arm choices that differed from the previous two choices ('successful choices') to total choices during the run ('total entry minus two' because the first two entries could not be evaluated). For example, if a rat made 10 entries, such as1-2-3-2-3-1-2-3-2-1, there are 5 successful choices in 8 total choices (10 entries minus 2).

The elevated-plus maze (EPM) apparatus was made of four crossed arms (Kawasumi et al., 2004; Itoh et al., 1990). Two arms were open (50 × 10 cm grey plastic floor plate without wall), whereas the other two were closed (same floor plates with 20-cm-high transparent acrylic wall). The maze was set at 100 cm above the floor. Rats were allowed to explore the maze freely for 90 s. Examined parameters were: (1) transfer latency (the time elapsed until the first entry to a closed arm); (2) duration of the first stay in a closed arm (the time from the first entry to a closed arm to the first escape from the arm); (3) cumulative time spent in the open/closed arms.

Water-finding task (WFT) was performed to analyze latent learning or retention of spatial attention of the rats (Kawasumi et al., 2004; Ichihara et al., 1989; Mamiya et al., 1998). The testing apparatus consisted of a grey plastic rectangular open field (50 × 30 cm, with a black 10-cm^2 grid) with a15-cm wall, and a cubic alcove (10 × 10 × 10 cm) was attached to the center of one longer wall. A drinking tube was inserted through a hole at the center of the alcove ceiling, with the tip of the tube placed at 5 cm for training or at 7 cm for the trial from the floor. A mouse was first placed at the near-right corner of the apparatus and allowed to explore it freely for 3 min. Rats were omitted from the analysis when they could not find the tube within the 3-min exploration. After the training session, rats were deprived of water for 24 h. In the trial session, rats were again individually placed at the same corner of the apparatus and allowed to find and drink the water in the alcove. The elapsed times until the first entry into the alcove (entry latency, EL), until the first touching/sniffing/licking of the water tube (contacting latency, CL) and until the initiation of drinking from the water tube (drinking latency, DL) were measured.

2.4. Histopathology

Liver tissues were fixed in 10% formalin for 24 h and then paraffin-embedded in an automated tissue processor; 5 μm sections were stained with Hematoxylin and Eosin (H&E) or Masson and subjected to histopathological examination.