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Increased Reissner's fiber material in the subcommissural organ and ventricular area in bile duct ligated rats

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ABSTRACT

Hepatic encephalopathy is a common neuropsychiatric complication of acute and chronic liver failure. Whether brain structures with strategic positions in the interface of blood–brain barriers such as the circumventricular organs are involved in hepatic encephalopathy is not yet established. Among the circumventricular organs, the subcommissural organ secretes a glycoprotein known as Reissner's fiber, which condenses and forms an ever-growing thread-like structure into the cerebrospinal fluid. In the present work we describe the Reissner's fiber material within the subcommissural organ and its serotoninergic innervation in an animal model of chronic hepatic encephalopathy following bile duct ligation in experimental rats. The study involved immunohistochemical techniques with antibodies against Reissner's fiber and 5-hydroxytryptamine (5-HT). Four weeks after surgical bile duct ligation, a significant rise of Reissner's fiber immunoreactivity was observed in all subcommissural organ areas compared with controls. Moreover, significant Reissner's fiber immunoreactive materials within the ependyma and inside the parenchyma close to the ventricular borders were also seen in bile duct ligated rats, but not in control rats. Increased Reissner's fiber material in bile duct ligated rats seems to be related to a reduction of 5-HT innervation of the subcommissural organ, the ventricular borders and the nucleus of origin, the dorsal raphe nucleus. Our data describe alterations of the subcommissural organ/Reissner's fiber material and the subcommissural organ 5-HT innervation probably due to a general 5-HT deficit in bile duct ligated rats.

Introduction

Hepatic encephalopathy (HE) is a common neuropsychiatric complication of both acute and chronic liver failure where ammonia and proinflammatory cytokines are thought to induce systemic-derived neurotoxicity (Shawcross and Jalan, 2005). Recently it has been suggested that among other mechanisms, cerebral inflammation is involved in HE, either due to chronic (Rodrigo et al., 2010) or acute (Jiang et al., 2009) liver failure. It has been shown that during systemic-CNS inflammatory processes, brain structures that are mostly affected are those located in strategic positions at the interface of the blood–brain barriers particularly the circumventricular organs (CVO) (Stitt, 1990; Lacroix et al., 1998). However, little is known about the role of these circumventricular organs in the pathophysiology of hepatic encephalopathy including the subcommissural organ (SCO).

The SCO is an ependymal structure located in the roof of the third ventricle at the entrance to the Sylvian aqueduct, and is formed by tall and elongated secretory ependymal cells (Reissner, 1860). The SCO ependymocytes contain particularly large cytoplasmic cisternae of endoplasmic reticulum filled with glycoproteins (Rodriguez et al., 1998). This secretory material is released mainly into the third ventricle, but also liberated at the basal part of the organ into the blood vessels, where part of it may condense and form a continuously growing thread-like structure known as Reissner's fiber (RF) that extends along the aqueduct, fourth ventricle and central canal of the spinal cord and ends in a dilatation known as the terminal ventricle (Rodriguez et al., 1998). The SCO secretory materials are N-glycosylated proteins with high mannose carbohydrate chains (Rodriguez and Yulis, 2001). Molecular components of the SCO material revealed several glycoproteins with a major constituent being the SCO-spondin of approximately 450 kd (Creveaux et al., 1998), which is present in several vertebrate species including: cattle, rat, mouse, chicken and zebrafish (Gobron et al., 1999). By addition of newly released glycoproteins to its cephalic end, the RF continuously grows in a rostro-caudal direction at a constant rate under the control of innervating systems of the SCO

Abbreviations: BDL, bile duct ligation; CSF, cerebrospinal fluid; CVO, circumventricular organs; DRN, dorsal raphe nucleus; HE, hepatic encephalopathy; 5-HT, 5-hydroxytryptamine; RF, Reissner's fiber; SCO, subcommissural organ.

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(Rodriguez et al., 1992). The SCO is a target for several innervations (Rodriguez et al., 1992), and previous studies have demonstrated that a serotoninergic system controls the RF material in several species (Léger et al., 1983; Sakumoto et al., 1984; Ahboucha et al., 2000; Laalaoui et al., 2001; Richter et al., 2004; Bermúdez-Silva et al., 2010).

Although the precise function of the SCO remains obscure, several roles have been attributed to this organ, including salt and water homeostasis, neuronal survival, regulation of neurotransmitter concentrations and detoxification of CSF (Monnerie et al., 1995; Caprile et al., 2003; Bouyatas and Gamrani, 2007; Elgot et al., 2009) as well as its possible role in the pathophysiology of lordosis and hydrocephalus (Ahboucha and Gamrani, 2001; Meiniel, 2007). So far, no study has been performed on the role of CVO in HE and the objective of the present study was to evaluate the extent of RF material within the SCO in cirrhotic rats. In an animal model of chronic HE due to bile duct ligation, we conducted immunohistochemical studies to evaluate the RF material and 5-HT innervation of the SCO together with the 5-HT system at the nucleus of origin, namely the dorsal raphe nucleus (DRN).

Materials and methods

Animals

Male Sprague-Dawley rats weighing 200–250 g were housed at a constant room temperature (22 °C), with a 12-h dark–light cycle and free access to food and water to all studied groups. All animals were treated in compliance with the guidelines of the Cadi Ayyad University, Marrakesh, Morocco. Rats were divided into two groups. *Group* 1: rats subjected to bile duct ligation surgery (BDL rats) ($n = 5$). Rats were anesthetized by inhalation of isoflurane then subjected to a midline abdominal incision, the bile duct was isolated, double-ligated, and resected between the ligatures as described by Cameron and Oakley (1932). *Group* 2: sham-operated controls ($n = 6$) which were subjected an abdominal incision and similar organ manipulation without bile duct ligation. Four weeks after BDL surgery, rats did not show a significant change of their body weight, but had a significant increase in liver weight, and the liver/body weight ratio as previously described (Butterworth et al., 2009a). At this stage, BDL rats had elevated bilirubin and elevated plasma liver enzymes (Butterworth et al., 2009a). Cirrhosis was confirmed by liver histology, which showed expected changes in the cytoarchitecture of the liver of BDL rats.

Locomotor activity

The "open field" test was used to evaluate locomotor activity of sham-operated ($n = 5$) and BDL rats ($n = 6$). The apparatus consists of 25 identical squares of 20 cm per side (100 cm × 100 cm × 40 cm) made out of wood. The animal is placed in the middle of the field and the ambulations (counting of the number of squares traversed by animal) were recorded for 5 min. For habituation of animals to the test, rats were exposed individually to the open field for 10 min on 3 consecutive days before the study.

Immunohistochemistry

Four weeks after surgery, cirrhotic rats ($n = 3$) and sham controls ($n = 3$) were anesthetized, and perfused transcardially with chilled physiological saline and paraformaldehyde (4%) in phosphate buffered saline (PBS, 0.1 M, pH 7.4). Brains were post-fixed in the same fixative overnight at 4 °C, dehydrated in graded ethanol solutions (50–100%), passed through serial polyethylene glycol (PEG) solutions and embedded in pure PEG. Frontal sections (20 μm) were cut with a microtome, collected and rinsed in PBS to wash out the fixative. Sections were taken throughout the SCO and the dorsal raphe nucleus (DRN). The immunolabelling was performed on six selected sections in the median part of these structures. Free floating sections were incubated in 5-HT (Miles, Elkhart, IN, USA) or polyclonal RF antibodies (Meiniel et al., 1996) respectively, diluted 1/5000 and 1/1000 in PBS containing 0.3% Triton X-100 and 1% bovine serum albumin. After three washes in the same buffer, sections were incubated for 2 h at room temperature in goat anti-rabbit biotinylated immunoglobulins (1/2000, Dako, Glostrup, Denmark) and then, after washing, incubated in streptavidin peroxidase (1/2000; Dako, Glostrup, Denmark). Peroxidase activity was visualized by incubating sections in 0.03% DAB (3-3-diaminobenzidine, Sigma–Aldrich, Oakville, Canada) in 0.05 M Tris buffer, pH 7.5, containing 0.01% H_2O_2. The sections were then collected, dehydrated and mounted in Eukitt® (Sigma–Aldrich, Buchs, Switzerland) for optical microscopy observation. The specificity of the immunoreactive materials was tested by treating slides to the same immunohistochemical protocol described above by either using the preimmune serum or omitting the primary antibodies. These controls showed that both primary antibodies used against RF and 5-HT display specific labeling as has been previously published by our group (Elgot et al., 2009).

Immunolabeling quantification

The quantification of the immunoreactive materials was performed according to the protocol published by Vilaplana and Lavialle (1999). Briefly, the digitization and storage of images were performed using a Zeiss-Axioskop 40 microscope (Carl Zeiss, Oberkochen, Germany) equipped with a Canon digital camera. Images were digitized into 512 × 512 pixels with eight bits of grey resolution and were stored in TIFF format. Image processing and quantification were performed using Adobe Photoshop v.6.0 (Adobe Systems, San Jose, CA, USA). After conversion of each image to the binary mode, the percentages of black pixels were obtained using the image histogram option of Adobe Photoshop. This percentage corresponds to the 5-HT or RF immunopositive areas of the ventricular borders and throughout the whole SCO including the apical and the basal parts and the fibers extending outside the organ. Five sections from sham-operated controls and BDL rats were randomly chosen for the quantification.

Statistical analysis

The data were expressed as mean ± SEM. Data were subjected to the Student *t*-test. A value of $P < 0.05$ was considered to indicate statistical significance between sham and BDL groups. Statistical analyses were performed using GraphPad Prism computer software (GraphPad Software Inc., San Diego, CA, USA).

Results

Locomotor performance

Locomotor performance in an open field test was done to evaluate the presence of encephalopathy in cirrhotic rats and was estimated as the number of boxes crossed by rats during 5 min. After a period of habituation, the BDL rats showed a significantly reduced number of crossed boxes compared to sham-operated animals (Fig. 1).

SCO/RF materials in periventricular area

RF immunoreactive material within the SCO

In sham-operated rats, the SCO showed RF-immunoreactivity which labeled several parts of the organ in particular within the