Changes in FABP1 and gastrin receptor expression in the testes of rats that have undergone electrical injury

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Abstract. Testicular trauma may occur due to accidental electrical injury. The aim of this study was to investigate alterations in the levels of fatty acid-binding protein 1 (FABP1) and gastrin receptor (gastrin R) in the testes following electrical injury. Sprague-Dawley rats were divided into control, fatal electrocution (220 V, 50 Hz, 60 sec) and electrical injury (220 V, 50 Hz, 60 sec) groups (n=8 per group). The animals in the fatal electrocution and electrical injury groups were deeply anesthetized with sodium pentobarbital prior to each treatment, in which the current was delivered via an anode connected to the left foreleg and a cathode to the right hindleg. The rats that survived were subsequently sacrificed by cervical dislocation. Control animals received cervical dislocation alone. Immunohistochemical analysis was performed to evaluate the protein expression of FABP1 and gastrin R in the testes. Sections were evaluated by digital image analysis. The expression levels of FABP1 and gastrin R were significantly increased following electrical injury, supported by an increase in the integrated optical density (IOD) when compared with that in the control group (P<0.05). However, no significant difference was found in FABP1 and gastrin R expression levels between the fatal electrocution and control groups. In summary, the protein expression levels of FABP1 and gastrin R were found to be significantly altered by electrical injury, suggesting that these two proteins may be important in underlying mechanisms of testicular injury during electrical injury. The findings indicate that such alterations would be reflected in abnormal testicular function.

Introduction

Electrical stimulation plays an important role in the cure of disease and relief of pain. It has been reported that patients with spinal cord injury (SCI) have many factors that are associated with pressure ulcer formation, including paralysis, loss of sensation, poor nutrition, anemia, and skin maceration associated with incontinence (1). Chronic pain in SCI is disabling and resistant to common pharmacologic approaches. It has been demonstrated that electrical and magnetic neural stimulation techniques are potential tools for use in the management of patients with this condition (2,3). The stimulation of leg and gluteal muscles during functional electrical stimulation cycling increases ankle excursion in individuals with spinal cord injury (4).

However, it has been shown that electrical injuries induce progressive tissue loss (5). Electrical injuries are devastating and are challenging to treat due to the complexity of the tissue damage and physiological impact (6). When the injury occurs at a high voltage, significant damage occurs to blood vessels via a number of mechanisms; the rupture of a major vessel is a rare but life-threatening sequela of electrical injury (7). Following high-voltage electrical injury, the endothelial and smooth muscle functions of the brachial artery are significantly decreased (8). In addition, electrical injury is reportedly a major cause of morbidity (9).

It has previously been shown that the effects of electrical injury include changes in the levels of type III collagen expressed in cardiac tissue (10); however, little is known about the changes in fatty acid-binding protein 1 (FABP1) and gastrin receptor (gastrin R) expression in testes that have undergone electrical injury.

The current study used immunohistochemistry to evaluate the expression of FABP1 and gastrin R in the testes of electrically injured rats, in order to determine whether their expression at the protein level is altered by electrical injury.

Materials and methods

Animal care. A total of 24 Sprague-Dawley rats, weighing 180-200 g, were provided by Sun Yat-Sen University (Guangzhou, China). All animals were given free access to standard pellet chow and water prior to the experiments. All
Animal treatment and study design. The 24 Sprague-Dawley rats were divided into three groups, namely a control group, a fatal electrocution group and an electrical injury group (n=8 per group). Sixteen of the rats were deeply anesthetized with sodium pentobarbital. A TMB 1000VA control transformer was used to provide electrical current via an anode and cathode (Zhejiang 001 Group Co., Ltd., Quzhou, Zhejiang, China). With the anode connected to the left foreleg and the cathode to the right hindleg, the rats were electrocuted (220 V, 50 Hz) for 60 sec. The 8 rats that died were defined as the fatal electrocution group, and the others were defined as the electrical injury group. The rats in the electrical injury group were sacrificed by cervical dislocation. In the control group, 8 rats were sacrificed by cervical dislocation without any prior electrical stimulus. Following sacrifice, the rat testes were rapidly excised and perfused with 10% neutral-buffered formalin solution (10).

Histopathological examinations. Specimens of the testes from each group were removed for histopathological examination. The testicular tissues were fixed in phosphate-buffered 4% formalin (pH 7.4) for 24 h and then embedded in paraffin. Sections were cut into 4-µm slices, and stained with hematoxylin and eosin. The slides were coded and semiquantitative analysis of the sections was performed by a pathologist who was blinded to the treatment that the rats had received. The pathological changes in these testicular tissues were then evaluated (11,12).

Tissue sections and immunohistochemical staining. All rat testes were immersed in 10% neutral-buffered formalin solution for 24 h, prior to being embedded in paraffin and sectioned with a microtome into 4-µm sections. All rat testes were investigated by immunohistochemistry to determine the difference between the electrical injury and control groups or the fatal electrocution and control groups with respect to FABP1 and gastrin R expression. The immunochemical analyses were performed as described previously (13-15). The primary antibodies were mouse monoclonal anti-FABP1 [sc-271591; L-FABP (F-9); Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA] and anti-gastrin R (BA2104; Wuhan Boster Biological Technology, Ltd., Wuhan, China), which were used at a dilution of 1:400.

The total integrated optical density (IOD), a parameter representing the expression levels of FABP1 and gastrin R in the testicular tissue, was determined using a Bx41 cast-grid microscope with DP10 camera (Olympus, Tokyo, Japan) together with an image-analysis program (MetaMorph offline, version 4.65; Molecular Devices, Sunnyvale, CA, USA). Under a magnification of x200, five images were examined in each immunostained section and the average IOD was calculated (15-17).

Statistical analysis. Results are expressed as mean ± standard error of the mean. The significance of differences in total IOD values was tested by Kruskal-Wallis analysis. P<0.05 was considered to indicate a statistically significant difference. All analyses were performed using SPSS software, version 12.0 (SPSS Inc., Chicago, IL, USA).

Results

Histological examination. Routine histological examination revealed little morphological change in the rat testes from each group (not shown).

Expression of FABP1 protein. The distribution of FABP1 in the rat testes of the control (Fig. 1A), fatal electrocution (Fig. 1B) and electrical injury (Fig. 1C) groups is displayed in Fig. 1. The total IOD for FABP1 in the testes of animals subjected to electrical injury (0.0134±0.00056) was significantly higher than that of the testes in the control group (0.0069±0.00035; P<0.05; Table I). The expression level of FABP1 in the control group was the lowest among the three groups; however, no significant difference in FABP1 expression was found between the fatal electrocution (0.0077±0.00041) and control groups.

Expression of gastrin R protein. The distribution of gastrin R protein in the rat testes of the control (Fig. 2A), fatal electrocution (Fig. 2B) and electrical injury (Fig. 2C) groups is displayed in Fig. 2. The total IOD of gastrin R in the testes of animals subjected to electrical injury (0.0126±0.00033) was significantly higher than that of control testes (0.0066±0.00048; P<0.05; Table I). The expression level of gastrin R in the control group was the lowest among the three groups; however, no significant difference in gastrin R expression was found between the fatal electrocution (0.0075±0.00052) and control groups.

Discussion

FABP1, also known as liver fatty acid-binding protein (L-FABP) has been demonstrated to be strongly associated with several diseases. FABP1 is a highly conserved key factor in lipid metabolism (18). The structures, functions and expression of a whole family of FABPs have been investigated extensively (19). Plasma and especially urinary levels of ileal bile acid binding protein (I-BABP) can improve the early diagnosis of intestinal ischemia, and plasma I-BABP levels can assist in

Table I. IOD of FABP1 and gastrin receptor in immunohistochemically stained rat testes.

<table>
<thead>
<tr>
<th>Groups</th>
<th>FABP1</th>
<th>Gastrin receptor</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>0.0069±0.00035</td>
<td>0.0066±0.00048</td>
</tr>
<tr>
<td>Fatal electrocution</td>
<td>0.0077±0.00041</td>
<td>0.0075±0.00052</td>
</tr>
<tr>
<td>Electrical injury</td>
<td>0.0134±0.00056*</td>
<td>0.0126±0.00033*</td>
</tr>
</tbody>
</table>

Results presented are the IOD per field, which is proportional to the total amount of staining. *P<0.05 vs. control group.
the localization of ileal ischemia (20). It has been suggested that L-FABP may have an important role in the prevention of age- or diet-induced obesity (21). A previous study has indicated that alterations in FABP1 may be associated with the development of aspirin-exacerbated respiratory disease (22). The FABP1 gene is highly transcribed in liver-derived cells, and regulated predominantly by liver-enriched transcription factors HNF3β and C/EBPα (23). A role of L-FABP in the attenuation of hepatic steatosis has also been demonstrated (24). SCP-2 expression has also been indicated to play a significant role in high density lipoprotein-mediated cholesterol efflux by regulating the size of rapid versus slow cholesterol efflux pools and/or eliciting the concomitant upregulation of L-FABP in cultured primary hepatocytes (25).

FABP1 serves as a key regulator of hepatic lipid metabolism, and FABP1 gene polymorphisms have been associated with several metabolic traits (26). It has also been shown that phosphorylation of Sar1b disrupts the FABP1-containing four-membered 75-kDa protein complex in the cytosol, enabling it to bind to the endoplasmic reticulum and generate pre-chylomicron transport vesicle (27). FABP1 plays an important role in the male reproductive system (28). In the present study, FABP1 expression was found to be significantly increased in electrically injured rat testes, indicating that elevated FABP1 expression is associated with testicular injury.

Gastrin is a peptide hormone, which regulates gastric acid secretion and exerts physiological actions such as the regulation of sodium balance (29). Gastrin has been suggested to be involved in blood pressure regulation, possibly via the regulation of sodium and water metabolism and/or the renin-angiotensin-aldosterone system (29). Measurements of gastrin levels are taken primarily for the diagnosis of gastrin-producing tumors (gastrinomas), which cause Zollinger-Ellison syndrome. Gastrin circulates as several bioactive peptides, and the peptide pattern in gastrinoma patients often deviates from that in normal individuals (30). Gastrin and its precursors have been shown to promote mitogenesis and angiogenesis in gastrointestinal tumors (31).

Gastrin-releasing peptide (GRP), which is an unfolded protein response regulator that functions as a Ca2+-binding molecular chaperone in the endoplasmic reticulum, is a regulatory human peptide that induces the release of gastrin and regulates gastric acid secretion and enteric motor function (32). GRP is a member of the bombesin-like peptide family. It has been reported that GRP stimulates the proliferation and invasiveness of androgen-independent prostate carcinoma (33). GRP mediates its action through the membrane-bound receptor, GRP receptor (GRPR), which is characterized by high-affinity binding for GRP and bombesin (33). GRPR is an attractive target for therapeutic and diagnostic applications, and is overexpressed in prostate cancer (34). The expression of GRPR is elevated in mucosa adjacent to head and neck squamous cell carcinoma (HNSCC) compared with mucosa from cancer-free controls, suggesting that elevated GRPR expression may indicate presence of HNSCC (35). GRP acts as a neuropeptide through G protein-coupled receptors involved in signal transmission in the central and peripheral nervous systems (36). It has also been proposed that GRPR is an alternative chemotactic receptor that may be involved in the pathogenesis of inflammatory disorders (36). In addition, gastrin plays an important role in the male reproductive system (37,38). In the current
study, it was revealed that the expression level of gastrin R following electrical injury was higher than that in the control testes, indicating that elevated gastrin R levels are associated with testicular injury.

In the present study, it was demonstrated that the levels of expression of FABP1 and gastrin R in the testes were altered in electrically injured rats. Following an electrical injury, the expression levels of FABP1 and gastrin R were significantly elevated in the rat testes, indicating an association with testicular trauma in rats undergoing electrical injury.

In conclusion, the current findings indicate that such alterations would be reflected in abnormal testis function.

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References