



Single-prolonged stress induces apoptosis in the amygdala in a rat model of post-traumatic stress disorder

Jinlan Ding, Fang Han, Yuxiu Shi *

Department of Histology and Embryology, Basic Medical College, China Medical University, Shenyang 110001, China

ARTICLE INFO

Article history:

Received 3 October 2008

Received in revised form 12 May 2009

Accepted 2 June 2009

Keywords:

Post-traumatic stress disorder

Amygdala

Apoptosis

Bax

Bcl-2

TUNEL

Flow cytometry

ABSTRACT

Objective: To detect the apoptosis-related Bax and Bcl-2 gene expression and apoptotic cell death in the amygdala region in the single-prolonged stress (SPS) rats.

Methods: A total of 100 male Wistar rats were randomly divided into a normal control group and SPS groups of 1d, 4d, 7d, and 14d. The expression of Bax and Bcl-2 was detected using immunohistochemistry and Western Blotting; TUNEL-staining and double-labeled flow cytometry (FCM) were employed for the detection and quantification of the apoptotic cells in the amygdala; morphological change of the subcellular structure in amygdala was observed by using the transmission electron microscopy (TEM).

Results: The ratio of Bax/Bcl-2 peaked at SPS 4d and then gradually decreased. The apoptosis peaked at SPS 4d. The TUNEL-positive cells were found in each SPS group and the TUNEL-positive cells rate peaked at SPS 4d. The morphological change of amygdala cells in each SPS group bears typical apoptotic characteristics.

Conclusions: In the SPS rat brain, we found apoptotic process in the amygdala region which may relate to the pathogenesis of amygdala abnormal function in PTSD.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Post-traumatic stress disorder (PTSD) is a stress-related mental disorder caused by traumatic experience. It exhibits four major types of characteristic symptoms: re-experiencing, avoidance, numbing, and hyperarousal (APA, DSM-IV, 1994). PTSD is thought to involve a dysregulation of medial prefrontal cortex and amygdala activity in response to fear. Amygdala, one of the key regions in the limbic system of the brain, has been documented to have an important role in emotional memory as fear or rage (Aggleton, 1992; LeDoux, 1995; McGaugh and Cahill, 1997).

A study of patients with PTSD related to early-age abuse (Bremner et al., 1997) showed a 15% smaller left amygdala volume than that of healthy subjects. Police officers with PTSD had smaller amygdala volumes (Lindauer et al., 2004). Matsuoka et al. (2003) found that the amygdala volume of PTSD is smaller than that of control subjects who have cancer but no PTSD. Meta-analyses showed left amygdala volume is significantly smaller in adults with PTSD compared with both healthy and trauma-exposed controls (Karl et al., 2006). Until today, no study has shown the cause of the amygdala volume decrease during PTSD. A relevant MRI study showed that significant hippocampal volume reduction due to combat or sexual abuse in the childhood (Lindauer et al.,

2005). Another study showed that the mechanism causing such smaller volume is apoptosis (Zhang et al., 2006). The amygdala has a close functional link to the hippocampus. Part of the hippocampus functions are regulated by amygdala (Roosendaal et al., 2003). In this paper, we have investigated the cause of the size and structure alternation of the amygdala after PTSD focused on apoptotic cell death.

Amygdala can be divided into three distinct subgroups: central nucleus, corticomедial nucleus, and basolateral nucleus (Harding et al., 2002). The basolateral nucleus is the largest among these three (Sims and Williams, 1990; Gloor, 1997). It is the key region of fear initiation. So, the basolateral nucleus has been paid great attention. Vyas and co-workers have reported chronic, unpredictable stress induces atrophy in bipolar neurons of basolateral amygdala (Vyas et al., 2002). We focus on observing the change of basolateral nucleus.

The Bcl-2 gene family plays a crucial role in the control of apoptosis. It includes pro-apoptotic proteins as Bax (Panaretakis et al., 2002; Bellosillo et al., 2002) and anti-apoptotic protein as Bcl-2 (Konopleva et al., 2002; Van Der Woude et al., 2002). Some in vitro experiments show that promote apoptosis by overexpression of Bax (Matter-Reissmann et al., 2002; Lanzi et al., 2001; Mertens et al., 2002; Mehta et al., 2002; Chang et al., 2002), while overexpression of Bcl-2 has been shown to inhibit apoptosis (Chen et al., 2002; Usuda et al., 2002; Sun et al., 2002; Jang et al., 2002; Tili et al., 2002). Thus, the Bax/Bcl-2 ratio appears to be a critical

* Corresponding author. Tel.: +86 13386887468; fax: +86 24 23256666x5305.
E-mail address: shiyuxiu@163.com (Y. Shi).

threshold parameter for apoptotic event (Jarskog et al., 2004; Cregan et al., 2004; Pettersson et al., 2002). Flow cytometry can be applied for the quantification of cell death; TUNEL-staining can demonstrate cellular apoptosis; the characteristic apoptotic morphological pattern can be detected by transmission electron microscopy.

To sum up, this study will assess the consequences of single-prolonged stress on:

- (1) The expression of Bax and Bcl-2.
- (2) The number of apoptotic cells.
- (3) The change of the number of TUNEL-positive cells.
- (4) Morphological changes in the amygdala cells.

2. Materials and methods

2.1. Animals

Male Wistar rats (China Medical university, $n = 100$, about 8 weeks old, weighing 150–180 g) were used for all experiments. All rats were reared in the experimental animal facility for a week to let them acclimate to their new environment (temperature: 18–20 °C, humidity: 50–60%, lights on: 07:00–19:00). Standard food pellets and tap water were available ad libitum. All procedures followed the National Guidelines on Animal Care.

2.2. Animal model and experimental groups

Animal models are powerful tools to elucidate the molecular mechanisms and the pathophysiology of mental disorders. There are several animal models for PTSD. Although these animal models present behavioral alterations resembling PTSD, they failed to show the most consistent neuroendocrinologic characteristic observed in PTSD patients (Yehuda et al., 1993; Stein et al., 1997; Yehuda, 2001, 2005). SPS is one of the animal models proposed for PTSD (Kohda et al., 2007). The SPS rats show enhanced inhibition of the hypothalamo–pituitary–adrenal (HPA) axis, which has been frequently demonstrated in patients with PTSD.

The SPS model consisted of 2 h whole body restraint in an animal holder, and was followed immediately by 20 min of forced swimming (temperature: 25 °C, depth: 40 cm). These rats were allowed to recuperate for 15 min. They were then exposed to ether vapor until loss of consciousness. The rats were put back into their home cage and left undisturbed until they were killed for experiments (Liberzon et al., 1999; Takahashi et al., 2006).

The rats were randomly divided into five groups: the SPS treatment groups (1d, 4d, 7d, and 14d) and the normal control group. The normal control rats lived in their individual home cages with no handling, and were killed after acclimating to their new environment for a week. Each group had 20 rats, in which five rats were used for paraffin sections, five for Western Blotting, five for flow cytometry, and five rats were used for TEM.

2.3. Fixation and sections making

Rats of both normal control and SPS groups were deep anesthetized with nembutal (30 mg/kg i.p.). The brains were removed after perfusion fixation with 4% paraformaldehyde (PFA) in 0.01 M PBS. Samples were dehydrated in alcohol and paraffin embedded. About 5 μm coronal sections were prepared for morphological studies.

2.4. Immunohistochemistry for Bax and Bcl-2

In order to detect Bax or Bcl-2-immuno-positive cells, one series of sections was stained by using the “PV two-steps”. The second

antibody polymerized with horseradish peroxidase into IgG-HRP. Then, the sections had color reaction directly after binding with primary antibody. Sections from each group were stained simultaneously to avoid introducing errors between groups. Dewaxed sections were washed three times (5 min each) in 0.01 M PBS. In order to increase the penetration of the Bax or Bcl-2 antibody, the sections were incubated in 0.01 M citrate buffer at 100 °C for 5 min, and washed again three times in 0.01 M PBS at room temperature. Then, the sections were incubated in 1:50 mouse polyclonal antibody against Bax (Santa Cruz Biotechnology) or 1:50 rabbit polyclonal antibody against Bcl-2 (Santa Cruz Biotechnology) overnight at 4 °C. After being washed with PBS, and then the sections were respectively, incubated with anti-mouse or anti-rabbit IgG-HRP antibody for 0.5 h at 37 °C. Finally, 3,3'-diaminobenzidine (DAB) was used as chromogen for 10 min until the brown color appeared. Slices were then dehydrated and mounted with neutral gum. To assess nonspecific staining, a few sections in every experiment were incubated in PBS without primary antibody.

Five slides were randomly selected from each group. Each slide was randomly selected five visual fields in basolateral amygdala ($\times 40$). We recorded the optical density (OD) of positive cells in each field to evaluate the average of OD, and then calculated the Bax/Bcl-2 ratio in the corresponding groups. The OD of immunoreactivity of Bax and Bcl-2-immunopositive cells were analyzed using a MetaMorph/DPIO/BX41 morphology image analysis system.

2.5. Double immunofluorescent labeling for Bax and Bcl-2

The sections were incubated with mouse polyclonal antibody against Bax (1:50) plus rabbit polyclonal antibody against Bcl-2 (1:50) overnight at 4 °C. After three times washing, the sections were incubated with CY3 anti-mouse IgG (1:1000) plus FITC anti-rabbit IgG (1:1000) for 0.5 h at room temperature. After being washed in PBS and mounted. Confocal laser scanning microscope was applied for colocalization observation.

2.6. Western Blotting for Bax and Bcl-2

Rats were decapitated, and the brain were removed and immediately placed in an ice-cold dish. Then the basolateral amygdala was dissected according to the atlas (Paxinos and Watson, 1998) by use of a stereomicroscope and was quick frozen in liquid nitrogen and stored at –80 °C. Amygdala of normal control rats and SPS rats were respectively, homogenized with a sample buffer containing 200 mM TBS, pH 7.5, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol and were denatured by boiling for 3 min. Samples (50 μg /lane) were loaded on a 7.5% SDS–polyacrylamide gel, and electroblotted onto a PVDF membrane (Millipore Corp., Bedford, MA) from the gel by a semi-dry blotting apparatus (Bio-Rad Laboratories, Inc., Hercules, CA).

The blotted membrane was then blocked with 1.5% skim milk, 0.05% Tween-20 in TBS (TBST) at 4 °C overnight, and then incubated with 1:50 mouse polyclonal antibody against Bax (Santa Cruz) or 1:50 rabbit polyclonal antibody against Bcl-2 (Santa Cruz) at 4 °C for 24 h. Blots were washed three times with TBST, and then incubated with a second antibody (anti-mouse or anti-rabbit IgG-HRP from Santa Cruz; 1:5000) for 2 h at room temperature. After incubation, blots were washed three times with TBST before visualization by enhanced chemiluminescence (ECL; Amersham Pharmacia Biotech). To confirm equal protein loading, the same blots were incubated with antibodies specific for β -actin (Abcam, British; 1:1000). Immunoreaction for β -actin was detected with the ECL.

The optical density (OD) of Bax, Bcl-2, and β -actin was analyzed on the Gel Image Analysis System (Tanon 2500R, Shanghai, PR Chi-

متن کامل مقاله

دریافت فوری ←

ISIArticles

مرجع مقالات تخصصی ایران

- ✓ امکان دانلود نسخه تمام متن مقالات انگلیسی
- ✓ امکان دانلود نسخه ترجمه شده مقالات
- ✓ پذیرش سفارش ترجمه تخصصی
- ✓ امکان جستجو در آرشیو جامعی از صدها موضوع و هزاران مقاله
- ✓ امکان دانلود رایگان ۲ صفحه اول هر مقاله
- ✓ امکان پرداخت اینترنتی با کلیه کارت های عضو شتاب
- ✓ دانلود فوری مقاله پس از پرداخت آنلاین
- ✓ پشتیبانی کامل خرید با بهره مندی از سیستم هوشمند رهگیری سفارشات