Journal of the Neurological Sciences 368 (2016) 307–313

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Effects of chronic noise on mRNA and protein expression of CRF family molecules and its relationship with p-tau in the rat prefrontal cortex

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ARTICLE INFO

Article history:
Received 29 May 2016
Received in revised form 15 July 2016
Accepted 21 July 2016
Available online 22 July 2016

Keywords:
Noise
Prefrontal cortex
Corticotropin-releasing factor
Corticotropin-releasing factor receptors
Phosphorylated tau

ABSTRACT

Chronic noise exposure has been associated with Alzheimer's disease (AD)-like pathological changes, such as tau hyperphosphorylation and β-amyloid peptide accumulation in the prefrontal cortex (PFC). Corticotropin-releasing factor (CRF) is the central driving force in the stress response and a regulator of tau phosphorylation via binding to CRF receptors (CRFR). Little is known about the CRF system in relation to noise-induced AD-like changes in the PFC. The aim of this study was to explore the effects of chronic noise exposure on the CRF system in the PFC of rats and its relationship to tau phosphorylation. Male Wistar rats were randomly divided into control and noise exposure groups. The CRF system was evaluated following chronic noise exposure (95 dB sound pressure level white noise, 4 h/day × 30 days). Chronic noise significantly accelerated the progressive overproduction of corticosterone and upregulated CRF and CRFR1 mRNA and protein, both of which persisted 7–14 days after noise exposure. In contrast, CRFR2 was elevated 3–7 days following the last stimulus. Double-labeling immunofluorescence co-localized p-tau with CRF in PFC neurons. The results suggest that chronic noise exposure elevates the expression of the CRF system, which may contribute to AD-like changes.

1. Introduction

Noise is becoming a widespread and daily source of stress in the living environments of modern societies. People are increasingly exposed to hazardous noise levels coming from many sources, including their work environment, urban traffic, the media, and household appliances [1,2]. The World Health Organization documented that noise affects mental health, decreases work capacity, induces sleep disturbances, and may be a risk factor for cardiovascular diseases [3]. Long-term noise exposure, in particular, is a health hazard, increasing the risk of physical damage [4]. Such exposure can have physiological or even pathological effects on the classical auditory system, as well as on non-lemniscal brain regions such as the hippocampus and cerebral cortex. Such exposure has been associated with the persistent tau and β-amyloid peptide (Aβ) pathology in the hippocampus and prefrontal cortex (PFC) that is observed in Alzheimer's disease (AD) [1,5], suggesting that chronic noise exposure might result in an increased risk of developing AD. However, the molecular mechanisms responsible for such noise-induced modifications in brain structures have not been established yet.

The physiological impact of stress is mediated by the hypothalamic-pituitary-adrenal (HPA) axis, a self-regulated pathway that utilizes its end product, corticosterone, to control its own activation through a negative feedback mechanism [6]. In response to stress, neurons of the hypothalamic paraventricular nucleus secrete corticotropin-releasing factor (CRF), which binds to its type 1 receptor (CRFR1) in the pituitary gland to activate the HPA axis. While physical stressors can directly activate the HPA axis, psychological stressors indirectly regulate the HPA axis, requiring higher-order sensory processing via specific brain structures, such as the hippocampus and the amygdala [7]. Noise is a physical and psychological stressor, exerting its effects on the HPA axis through these structures by connections with the auditory system [8].

The CRF system plays a prominent role in the coordination of neuroendocrine and neuropsychiatric responses to stress. The CRF system comprises CRF and its receptors, CRFR1 and CRFR2. CRF and its receptors are found in the hippocampus and PFC, and the dysregulation of the CRF system in these regions cause alterations in AD-like pathology in animals [9–12]. Supporting a role for CRF in AD neuropathology, work from many laboratories has demonstrated that both CRF overexpression and acute or repeated exposure to stressors induce phosphorylated tau (p-tau) and accumulation of Aβ within the hippocampus, a process that

Abbreviations: AD, Alzheimer disease; BSA, bovine serum albumin; CRF, corticotropin-releasing factor; CRFR1/2, Corticotropin-releasing factor receptor 1/2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HPA, hypothalamic-pituitary-adrenal; p-tau, phosphorylated tau; PFC, prefrontal cortex; TBS, Tris-buffered saline.

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http://dx.doi.org/10.1016/j.jns.2016.07.049
0022-510X/

is dependent on CRFR1 [9,11,13,14]. However, only limited information is available on the control of this system in the PFC.

CRFR1 and CRFR2 are widely but heterogeneously distributed in the central nervous system [15,16], suggesting distinctive functional roles for each receptor subtype. A prominent role for CRFR2 has been suggested in the regulation of anxiety-like behaviors after stress [17–20], but whether CRFR2 participates in AD-like responses to stress remains unclear.

Although the involvement of the CRF signaling system in AD-like changes induced by restraint or other stressors has been widely documented [9,11,13], the molecular mechanisms responsible for changes in this system following noise exposure have not been previously studied. In this study, we aimed to investigate the effects of noise stress on CRF system molecules and to explore the relationship between the CRF system and long-term noise-induced AD-like changes in rat PFC. For this purpose, mRNA and protein expression levels of CRF, CRFR1, and CRFR2 and the co-localization of CRF and p-tau in the PFC were analyzed after chronic white noise exposure.

2. Materials and methods

2.1. Animal use and experimental grouping

In total, 64 male 8-week-old Wistar rats (Lab Animal Center, Institute of Health and Environmental Medicine, Tianjin, P.R. China), weighing 200–220 g, were used in this study. The rats were kept in a room with a 12-hour light/dark cycle (with lights on from 06:00 to 18:00) and controlled ambient temperature (23 ± 2 °C) and humidity (50–70%). The rats had free access to water and food in their cages. The rats were habituated to the laboratory environment for 5 days prior to the start of the experiment, in which they were randomly assigned to either the noise-exposed or control group. Animals in the noise-exposed group were exposed to noise in a reverberation chamber with 95 dB sound pressure level white noise (4 h per day for 30 days, from 8:00 to 12:00). The animals were in wire-mesh cages placed in the center of the sound field, with one animal per cage. The loudspeaker was suspended directly above the cages. Rats in the control group were housed in similar cages but were exposed to background noise (below 40 dB sound pressure level) from another chamber. At different time points (days 0, 3, 7, or 14) after the final exposure, rats in the noise-exposed and control groups were sacrificed under chloral hydrate anesthesia (10%, 0.3 mL/100 g) for subsequent biochemical analyses (n = 8 rats per group and time point). Two rats from each time point and group were used for immunofluorescence, and 6 rats were used for ELISA, RT-PCR, and western blot analyses. Furthermore, two cortical tissue samples were isolated from the same part of the prefrontal cortex in each animal for RT-PCR and western blot analyses, respectively. All experiments were performed in accordance with approved guidelines specified by the Animal and Human Use in Research Committee of the Tianjin Institute of Health and Environmental Medicine.

2.2. Noise exposure apparatus

White noise was generated using a noise generator (BK 3560C, B&K Instruments, Denmark), amplified with a power amplifier (YONGSHENG AUDIO P-150D, The Third Institute of China Electronic Technology Group, China), and delivered through a loudspeaker (ZM-16 S, Tianjin Zenmay Electroacoustic Equipment Co., Ltd., China). The main spectrum of the noise emitted from the speaker was in the range of 400–6300 Hz (1/3 octave). All exposures were performed as described in our previous study [5].

2.3. Determination of plasma corticosterone by ELISA

Rats were sacrificed at the time points indicated under Section 2.1, and blood samples were collected in tubes containing heparin sodium as an anticoagulant. Samples were centrifuged at 4 °C, and after separation, the plasma was stored at −80 °C until assayed using a corticosterone ELISA kit (BlueGene Biotech, Shanghai, China), according to the manufacturer's instructions.

2.4. Determination of gene expression by real-time PCR

PFCs from noise-exposed and control rats were removed after the animals were sacrificed under chloral hydrate anesthesia, at the time points indicated in Section 2.1, and homogenized using a rapidly oscillating masher. Total RNA was extracted using an RNeasy Mini kit (TaKaRa Bio, Dalian, China), according to the manufacturer's protocol. Total RNA was converted to cDNA by reverse transcription using a Transcriptor First Strand cDNA Synthesis Kit (TaKaRa Bio, Dalian, China). Specific primers and probes designed for rat glyceraldehyde phosphate dehydrogenase (GAPDH; internal control), CRF, CRFR1, and CRFR2 sequences were used, as described in Table 1. Gene expression levels for CRF and CRFR1/2 were assessed by quantitative real-time PCR performed under the following thermal cycling conditions: 2 min at 50 °C, 10 min at 95 °C, and 45 cycles of 95 °C for 5 s followed by 57 °C for 30 s. Real-time PCR was performed using gene expression assays-on-demand and a Takara PCR Thermal Cycler Dice Real Time system (TaKaRa Bio, Dalian, China). Target gene transcript levels were calculated after normalizing cycle thresholds (Ct) to GAPDH expression and are presented as fold-induction values ($2^{-\Delta\Delta Ct}$, $\Delta Ct = Ct_{CRF/CRFR1/CRFR2} - Ct_{GAPDH}$, and $\Delta\Delta Ct = \Delta Ct_{exposure} - \Delta Ct_{control}$) relative to those of control rats.

2.5. Western blot analysis

The PFC was dissected immediately and homogenized in ice-cold radioimmunoprecipitation assay buffer (50 mM Tris–hydrochloric acid, pH 7.4, 1% Triton X-100, 0.2 mM phenylmethanesulfonyl fluoride, and 1 mM ethylenediaminetetraacetic acid). Homogenates were centrifuged at 14,000 × *g* for 15 min at 4 °C, and the supernatants were collected. Protein concentration was determined using the Bio-Rad protein assay with bovine serum albumin (BSA) (Sigma, USA) as a standard. Proteins were then denatured in boiling water for 10 min. Samples (20 μg protein/lane) were run on 10% sodium dodecyl sulfate polyacrylamide gels and transferred to microporous polyvinylidene difluoride membranes (0.45 μm, F. Hoffmann-La Roche Ltd., Germany) for 25 min at 12 V in a semi-dry blotting apparatus (DYPC-40C, Beijing Liuyi Instrument, China). Membranes were blocked with Tris-buffered saline (TBS) containing 2% (w/v) BSA and 0.5% Tween 20 (Sigma) for 1 h, incubated with primary antibodies for 12 h at 4 °C, washed in TBS with 0.1% Tween 20, and then incubated with peroxidase-conjugated Affinipure goat anti-rabbit IgG secondary antibodies (1:10,000, ZSGB-BIO, Beijing, China) for 1 h at room temperature. After visualizing with an enhanced chemiluminescence system (EMD Millipore Co., USA), the integrated intensity values of the immunoreactive signals were analyzed using Gel-Pro 3.1 software (Media Cybernetics Inc., USA).

Table 1
Rat primers used for real-time RT-PCR.

Gene	Primers
CRF	F: 5′-CGCCCATCTCTCTGGATCT-3′ R: 5′-TCTCCATCAGTTTCCTGTTGC-3′
CRFR1	F: 5′-GAACCTCATCTCGGCTTTCA-3′ F: 5′-GGCTGTCACCAACCTACACC-3′
CRFR2	F: 5′-TCATCCTCGTGCTCCTCATC-3′ R: 5′-GCCTTCACTGCCTTCCTGTA-3′
GAPDH	F: 5′-CAGGGCTGCCTTCTCTTGTG-3′ R: 5′-GATGGTGATGGGTTTCCCGT-3′

CRF, corticotropin-releasing factor; CRFR1, corticotropin-releasing factor receptor 1; CRFR2, corticotropin-releasing factor receptor 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.