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Research report

Sex-related differences in striatal dopaminergic system after traumatic brain injury

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ABSTRACT

Several studies have demonstrated alterations in the dopamine (DA) system after traumatic brain injury (TBI). Additionally, the existence of significant sex-related differences in the dopaminergic system has long been recognized. Accordingly, the purpose of the present study was to investigate whether TBI would differentially alter, in female and male mice, the expression and the function of the striatal vesicular monoamine transporter-2 (VMAT-2), an important DA transporter. After controlled cortical impact (CCI) injury, female mice showed significantly lower striatal DA concentrations and K^+-evoked DA output. By contrast, no significant sex-related differences were observed in the mRNA and protein levels of striatal dopamine transporter (DAT) and VMAT-2 and the methamphetamine (MA)-evoked DA output. These results demonstrated clear sex-related differences in striatal VMAT-2 function in response to TBI and suggested that female mice may be more sensitive to the TBI-induced inhibition of the VMAT-2 function, as indicated by the greater degree of deficits observed when the VMAT-2 DA-storage function was inhibited by TBI. Moreover, the TBI-induced suppression of locomotion was more pronounced than female mice. Such findings highlight the need for sex-specific considerations when examining differences among brain injury conditions.

1. Introduction

Each year, more than ten million people who suffer from traumatic brain injury (TBI) need medical attention (Hyder et al., 2007). In survivors, neuronal injury can lead to neurodegenerative diseases, which may be due to the altered dopaminergic system (Bales et al., 2009). Previous studies indicated that striatal dopamine (DA) concentration increased at acute time points after experimental TBI (Massucci et al., 2004; McIntosh et al., 1994). Additionally, DA transporter (DAT), which plays a key role in terminating the action of DA by rapid reuptake of DA from the synaptic cleft into the presynaptic terminals and thus maintaining DA homeostasis in the central nervous system, has also been found to be altered after TBI. For example, striatal DAT expression was reduced in male mice after controlled cortical impact (CCI) injury (Wagner et al., 2005b). Also, decrease in striatal DAT binding has been documented in patients 4–5 months after TBI (Donnemiller et al., 2000).

The existence of significant sex-related differences in the striatal dopaminergic system has been well established. For instance, striatal DAT density/number is higher in female than male rats (Rivest et al., 1995), and striatal DAT function assays indicate that a more active DAT is present in female than male rats (Walker et al., 2000). Similar results were observed in clinical studies reporting that healthy adult women had higher DAT density than men when measured by single photon emission computed tomography (SPECT) (Lavalaye et al., 2000). Moreover, sex-related differences can also influence the striatal dopaminergic system after TBI. For example, TBI induces chronically larger relative decreases in DAT expression in male than female mice (Wagner et al., 2005a). Such sex-related differences in the DAT expression and function may be associated with the salient differences between males and females in response to DAT inhibitor (Wagner et al., 2007).

Another important transporter related with striatal DA function is the vesicular monoamine transporter-2 (VMAT-2). The VMAT-2 sequesters cytoplasmic DA and thus prevents oxidation of DA in the cytoplasm. Additionally, VMAT-2 can also sequester neurotoxins within vesicles (Brown et al., 2006). In this way, VMAT-2 can function not only as a reservoir for DA, but also to protect DA neuron against neurotoxins. During normal dopaminergic neuro-

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transmission, the DAT regulates the extracellular concentration of DA, while VMAT-2 directly regulates the cytosolic concentration of DA and indirectly regulates the extracellular concentration of DA by determining the amount of DA released from the vesicular stores. Accordingly, these two transporters play critical roles in the striatal dopaminergic system by regulating the availability and access of intracellular and extracellular DA. The possession of more active VMAT-2/DA storage capacity by female mice has been attributed to sex-related differences in VMAT-2 (Ji et al., 2007). Moreover, the function of these two transporters may be linked and their interaction may be influenced by sex-related differences (Ji et al., 2009). However, whether these sex-related differences have an impact on VMAT-2 after TBI has not been fully elucidated.

Given the important influence of sex-related differences on the striatal dopaminergic system, a key mediator in post-TBI function and cognitive deficits, we hypothesize that TBI may impact the striatal dopaminergic system by altering striatal VMAT-2 in a sexually dimorphic manner. Therefore, the focus of the present study was to analyze the effects of sex-related differences on striatal the VMAT-2 expression level, DA concentration and DA release upon potassium and methamphetamine (MA) stimulation after injury. The comparison of the DA response to potassium and MA can serve to ascertain the potential mechanistic differences that exist between sexes and which might be present in the CCI model. In addition, sex-related differences in locomotor activity after CCI were also examined.

2. Materials and methods

2.1. Animals

Young adult (2–3 months) CD-1 male and female mice obtained from the Experimental Animal Central of Nanjing Medical University were used in our experiments. Female and male mice were randomized to TBI groups and sham-injured groups, separately. In these experiments, comparisons between TBI groups and sham-injured groups in male and female can indicate the role of sexual dimorphism in experimental TBI. While estrous cycle stage may affect the parameters to be measured, basic sex-related differences in dopaminergic function remain present (Dluzen and Horstink, 2003), and it is the evaluation of these basic sex differences that serve as the aim of this study, so the estrous cycle of the females were not recorded. All mice were individually caged and housed in a temperature-, humidity-controlled and pathogen-free room under a 12 h day-night cycle with food and water available ad libitum. All procedures followed the protocols which were approved by the Animal Care and Use Committee of Nanjing Medical University and in line with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All data were measured at one day after surgery. Every attempt was made to reduce the number of animals used and their suffering.

2.2. Experimental TBI model

The CCI model was used for the TBI groups as previously described (Dixon et al., 1991). The CCI procedure was performed while mice were anaesthetized with 4% isoflurane in a 2:1 N_2O/O_2 mixture and maintained by 1–1.5% isoflurane. Briefly, each mouse was mounted on a stereotaxic frame, a parasagittal craniotomy was performed over the left parietal area. For the TBI groups, the exposed dura was struck at approximately 5 mm lateral to the central suture at 4 m/s with a 2.0 mm tissue deformation by the pneumatic impactor. Immediately after the impact, the surgical area was sutured closed and the mice were returned to their cages to recover from anesthesia. The mice core body temperature was maintained at 37 °C until they recovered from anesthesia. Post-surgical righting reflex, a measure shown to be sensitive to the severity of the brain injury, was monitored. The sham-injured groups received the same procedures, except for the impact.

2.3. Polymerase chain reaction (PCR) analyses of DAT and VMAT-2 mRNA

Ipsilateral striatum total RNA was extracted from each group using the TRIzol agent (Invitrogen Corporation, Carlsbad, CA, USA) following the manufacture's instructions. The quantification and quality of the RNA were determined with of a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA). The cDNA was synthesized from 2 μg of total RNA using a reverse transcription system (Promega Corporation, Madison, WI, USA). Real-time PCR analysis was performed using the SYBR Green qPCR kit (Invitrogen Corporation). The primers for the PCR were as follows: DAT: forward 5′-TGGGCC TCAATGACACCTTT-3′ and reverse 5′-AGCAGAACAATGACCAGCACCA-3′; VMAT2: forward 5′-CTGTTCATCGTGTTCCTTGC-3′ and reverse 5′-AGAAGATGCTTTCGGAGGTG-3′; β-actin: forward 5′- AGCCATGTACGTAGCCATCC-3′ and reverse 5′-CTCTCAGCTGTGGTGGTGAA-3′. The mRNA expression level was normalized to β-actin using the standard $2^{-\Delta\Delta Cq}$ method.

2.4. Western blot analysis

For Western blot analysis, the frozen ipsilateral striatum tissue samples were completely homogenized, centrifuged at 14000*g* for 15 min at 4 °C, and then the supernatant was collected. The bicinchoninic acid (BCA) assay kit (Pierce, RocKford, IL, USA) was used to measure the protein concentration. Aliquots containing of 50 μg of VMAT-2 and 100 μg of DAT proteins from each sample were mixed with 2× sample buffer and boiled for 5 min. The proteins were resolved on a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred to a Hybond-polyvinylidene difluoride (PVDF) membrane. Antibodies were used for the detection of DAT (1:500; ab111468, Abcam, Cambridge, UK) and VMAT-2 (1:1000; sc-7721, Santa Cruz Biotechnology, Santa Cruz, CA, USA). The optical density of the resultant bands were analyzed using the ImageJ software (National Institutes of Health, Bethesda, MD, USA), and the densitometry measures were normalized to the β-actin signal.

2.5. Tissue selection, dissection and preparation

One day after surgery, mice were rapidly killed by rapid decapitation and the brain was quickly removed. Following a midline bisection, the ventricles on the medial side of the brain were pried open and the cortex cut away revealing the corpus striatum (CS). The ipilateral striatum was placed in 500 μL cold (4 °C) 0.1 N perchloric acid, sonicated and centrifuged at 15,000*g* for 20 min. An aliquot was removed for assaying the level of DA levels using a High-performance liquid chromatography (HPLC) system. K^+ induced and MA induced DA from the ipsilateral striatum of male and female mice in the TBI and sham-injuried groups were compared. The ipsilateral striatum was sliced into smaller tissue fragments and placed into cold (4 °C) Kreb's ringer phosphate (KRP) medium, and then in vitro superfusion was conducted. Given that the K^+ induced DA release from vesicular stores is a Ca^{2+}-dependent process, the increased DA response to K^+ infusion could be explained by a greater stored DA within vesicles. On the other hand, the MA induced DA output primarily involves a Ca^{2+}-independent alteration in the function of DAT. In this way, we can assess whether these two different mechanisms are influenced as a function of the mice conditions.