TRAUMATIC BRAIN INJURY RESULTS IN A CONCOMITANT INCREASE IN NEOCORTICAL EXPRESSION OF VASOPRESSIN AND ITS $V_{1a}$ RECEPTOR

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Arginine vasopressin (AVP) has been shown to promote the disruption of the blood-brain barrier (BBB) and the formation of edema in various animal models of brain injury. However, the source(s) of this AVP have not been identified. Since the cerebral cortex was considerably affected in some of these brain injury models, we sought to determine if AVP was produced in the cerebral cortex, and, if so, whether or not this cortical AVP expression was up regulated after injury. In the present study, a controlled cortical impact model of traumatic brain injury (TBI) in rats was used, and the temporal changes in expression of AVP and its $V_{1a}$ receptor were analyzed by real-time reverse-transcriptase polymerase chain reaction. The expression of AVP and its $V_{1a}$ receptor in the ipsilateral cortex adjacent to the lesion area was significantly up regulated between 4 h and 1 day post-TBI. The maximum increase in mRNA for AVP (4.3-fold) and its receptor (2.6-fold) in the ipsilateral vs. contralateral cortex was observed at 6 h post-TBI. Compared to sham-injured rats, no statistically significant changes in expression of AVP or its receptor were found in the contralateral cortex. These results suggest that the cerebral cortex is an important source of AVP in the injured brain, and the parallel increase in the expression of AVP and its cognate receptor may act to augment the actions of AVP related to promoting the disruption of the BBB and the formation of post-traumatic edema.

Key words: vasopressin, $V_{1a}$ receptor, traumatic brain injury, cerebral cortex, rat

INTRODUCTION

Arginine vasopressin (AVP) is involved in the regulation of fluid homeostasis in the normal brain (1-5). Over the years, compelling experimental evidence has also accumulated defining an important role for AVP in promoting the disruption
of the blood-brain barrier (BBB) and the formation of edema in several animal models of brain injury, such as cryogenic brain injury, focal cerebral ischemia, and intracerebral hemorrhage (6-11). In these studies, AVP-deficient Brattleboro rats or AVP-competent animals treated with the selective V$_{1a}$ receptor antagonists were used, which indicated that V$_{1a}$ receptors mediated the pathological actions of AVP in the injured brain. Centrally administered AVP has also been shown to produce edema in uninjured brain (12), and to exacerbate the post-ischemic edema formation in Brattleboro rats (6). These findings are consistent with clinical observations where patients with traumatic brain injury (TBI), cerebral ischemia, and subarachnoid hemorrhage, have had elevated AVP concentrations in the cerebrospinal fluid (CSF) and plasma (13-17).

The cerebral cortex was considerably affected in various models of brain injury, which raised an important question about the origin of AVP in this part of the brain. The sites of central AVP synthesis have been the topic of debate. A significant source of central AVP is the hypothalamus (18), where an increase in the expression of the AVP gene was observed after ischemic brain injury (19). AVP-containing axonal processes, originating in the hypothalamus, are located close to the lateral ventricles (20), suggesting that they may be a source of the AVP found in the CSF. AVP may also be released into the CSF from the choroid plexus epithelium, where it is synthesized (21). However, in the cerebral cortex, a limited number of AVP-immunoreactive axonal fibers have been found, and they appeared to only be localized to certain cortical areas (18). Furthermore, although volume transmission may play a role in distributing CSF-borne AVP within the brain, the penetration of this neuropeptide into the injured cortex is rather limited (22). Accordingly, the aim of this study was to determine if AVP was synthesized in the cerebral cortex, and, if so, whether or not this synthesis was up regulated after TBI. We also analyzed the time course of post-TBI changes in cortical expression of the V$_{1a}$ receptor to determine if changes in receptor expression correlated with those found for its ligand.

**MATERIALS AND METHODS**

*Animals and the TBI model.* Adult male Long-Evans rats weighing 280-320 g were purchased from Harlan Sprague Dawley (Indianapolis, IN, USA). They were kept at 22°C with a 12-h light cycle and maintained on standard pelleted rat chow and water *ad libitum*. All surgical and animal care procedures were in accordance with the guidelines of the Animal Care and Use Committee of Rhode Island Hospital and conformed to international guidelines on the ethical use of animals. Rats were anesthetized with intraperitoneal pentobarbital sodium (60 mg/kg). Rectal temperature was continuously monitored and maintained at ~37°C. The animals were placed in a stereotaxic frame, and a 4-mm craniotomy was performed on the right side of the skull to expose the dura, with the center of the opening located 3 mm posterior to bregma and 2.5 mm lateral to the midline. TBI was produced using a controlled cortical impact device obtained from Grzegorz Michałowski (Michalowski Inc., Zossen, Germany). The impact velocity was 5 m/s and its duration was 50 ms. The diameter of the impactor was 2.5 mm and the depth of brain deformation was set at 3 mm.
Immediately after TBI, the scalp was closed with a silk suture and the animals were allowed to recover in their cages. In sham-injured animals, the same surgical procedures were performed, but the brain was not injured.

Real-time reverse-transcriptase polymerase chain reaction (RT-PCR). At 4 and 6 h, and at 1 and 2 days after TBI, rats (4 animals per time point) were reanesthetized with intraperitoneal pentobarbital sodium, and the samples of the parietooccipital cortex from the area adjacent to the lesion and from the contralateral side were collected. Cortical samples from sham-injured rats were also collected. Total RNA was isolated using NucleoSpin RNA II kit (Macherey-Nagel, Düren, Germany). First-strand cDNAs were synthesized using oligo(dT)$_{20}$ primer (50 pmol) and 15 U of ThermoScript RNase H– reverse-transcriptase (Invitrogen, Carlsbad, CA, USA). Forty units of the RNase inhibitor, RNaseOut (Invitrogen), were also added to the reverse transcription reactions. For each 20-µl reaction, 1 µg of total RNA was used. The reaction was carried out for 1 h at 50°C.

The following primers and TaqMan probes were used: 5’-CCGAGTGTGAGGAGGTTTT-3’ (forward primer for AVP), 5’-CGGGAGCAGAGCAACGCACC-3’ (probe for AVP), 5’-CGACACAGCAAGGGTGAAGAAGG-3’ (forward primer for the $V_{1a}$ receptor), 5’-AGGAAGGCAAGCGCCCGG-3’ (reverse primer for the $V_{1a}$ receptor), 5’-CCCATTTGTGTGACGGCTTGAAGAC-3’ (probe for the $V_{1a}$ receptor), 5’-GGTAGAAAGGGCATGAGCA-3’ (forward primer for cyclophilin A), 5’-GCTACAGAAGGAATTGGTTGATG-3’ (reverse primer for cyclophilin A), and 5’-TTTGGGTCCAGGAATGGCAACGC-3’ (probe for cyclophilin A). Cyclophilin A was used for the normalization of mRNA for AVP and the $V_{1a}$ receptor. The predicted sizes of PCR products were 135, 265, and 152 bp for AVP, the $V_{1a}$ receptor, and cyclophilin A, respectively. Real-time PCR was performed using the DNA Engine Opticon System (MJ Research, Waltham, MA, USA). The 50-µl PCR reaction mixtures contained 0.2 mM mixed dNTPs, 0.2 µM each primer, 0.1 µM probe, 5 mM MgCl$_2$, 1 U HotStart Taq DNA polymerase (Qiagen, Valencia, CA, USA), and 1/20 (AVP or the $V_{1a}$ receptor) or 1/2000 (cyclophilin A) of the reverse transcription reaction product. The reaction mixtures were heated to 95°C for 15 min and then were subjected to 40 cycles of denaturation (96°C, 15 s), annealing/extension (60°C, 45 s) for AVP; denaturation (94°C, 15 s), annealing/extension (67°C, 1 min) for the $V_{1a}$ receptor; and denaturation (94°C, 15 s), annealing/extension (60°C, 45 s) for cyclophilin A.

Statistical analysis. The results of real-time RT-PCR are presented as mean number of copies of mRNA for AVP and the $V_{1a}$ receptor per 100 copies of cyclophilin A mRNA ± SEM. For statistical evaluation of data, ANOVA was used, followed by the Newman-Keuls test for multiple comparisons among means. P<0.05 was considered statistically significant.

RESULTS

The real-time RT-PCR analysis of changes in AVP expression in the ipsilateral parietooccipital cortex adjacent to the lesion area demonstrated that, within the period of time between 4 h and 1 day post-TBI, there was a significant increase in AVP mRNA compared to the similar cortical region in the contralateral, uninjured hemisphere or in sham-injured rats (Fig. 1A). AVP expression in the injured cortex was also elevated at 2 days post-TBI, but this increase was not statistically significant. The increase in mRNA for AVP was paralleled by the up-regulation of the $V_{1a}$ receptor expression. In the traumatized cortex, significantly elevated levels of the $V_{1a}$ receptor mRNA were observed between 4 h and 1 day
following TBI; at 2 days post-TBI, the number of transcripts for the $V_{1a}$ receptor in the injured cortex did not differ from those found in the contralateral cortex or in the cortex sampled from sham-injured rats (Fig. 1B). The maximum increase
in mRNA for AVP (4.3-fold, P<0.01) and its receptor (2.6-fold, P<0.01) in the ipsilateral vs. contralateral cortex was observed at 6 h after TBI. Compared to sham-injured rats, no statistically significant changes in the expression of AVP or its receptor were found in the contralateral cortex.

**DISCUSSION**

This study demonstrated that after TBI, there is a rapid (within 4 h) and relatively short-lasting (up to 1 day) up-regulation of AVP expression in the injured cortex adjacent to the lesion area. These results suggest that the increased cortical synthesis of AVP plays a role in the AVP-mediated disruption of the BBB and the exacerbation of edema observed after brain injury. The cellular origin of AVP in the cerebral cortex is presently unclear, but it is unlikely that this neuropeptide is produced by neurons. Indeed, using *in situ* hybridization histochemistry, Hallbeck *et al.* (23) have not been able to find AVP-producing neurons in the rat cerebral cortex, and the immunohistochemical analysis of the rat brain has shown only a small number of AVP-positive axonal fibers located in certain cortical areas (18). It is possible that the cortical AVP is synthesized by the endothelium of brain microvessels. Consistent with this idea, AVP has been found to be produced by the endothelia of large cerebral arteries (24) and various peripheral blood vessels (25 - 27), and this endothelial AVP synthesis is regulated by pathophysiological stimuli (27).

In parallel with increased AVP synthesis, we also observed an up-regulation of expression of the V₁a receptor in the cerebral cortex surrounding the lesion area. The time course of changes in the V₁a receptor expression was similar to that found for AVP, suggesting that this increase in expression of a cognate AVP receptor may augment the actions of AVP related to promoting the disruption of the BBB and the formation of edema in the injured brain. The present results are comparable with those previously obtained by us in a rodent weight-drop model of TBI (28). In this earlier study, the increased V₁a receptor expression was predominantly found in cortical astrocytes, particularly in those of a reactive, hypertrophic type, with elevated levels of expression of glial fibrillary acidic protein. Importantly, injury not only increased the astroglial expression of V₁a receptors, but also resulted in their redistribution from the astrocytic cell bodies to the astrocytic processes. Since the astrocytic processes form a close contact with the cerebrovascular endothelium, it is possible that in the injured brain, endothelium-derived AVP can act on astroglia in a paracrine manner. To test this hypothesis, further studies will be required.

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