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Short communication

Increased ornithine decarboxylase activity and protein level in the cortex following traumatic brain injury in rats

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Abstract

There is increasing evidence that the elevated levels of polyamines play an important role in the secondary injury following traumatic brain injury (TBI). Ornithine decarboxylase (ODC) is the rate-limiting enzyme of polyamine biosynthesis. Presently, we measured the ODC protein levels by Western blot analysis in the cerebral cortex of rats sacrificed at 2 h, 6 h, 24 h, 72 h and 168 h after controlled cortical impact injury. TBI resulted in a significant increase in ODC protein levels (2.5 to 5.5 fold, $P < 0.05$) and enzyme activity (13 to 21 fold, $p < 0.01$) between 2 and 6 h after the injury. ODC protein levels and enzyme activity returned to normal, control levels by 72 h after the injury. Increased ODC protein and enzyme activity could contribute to vasogenic edema and the pathogenesis of neuronal dysfunction after TBI by stimulating the formation of polyamines. © 1998 Elsevier Science B.V.

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Polyamines (putrescine, spermine and spermidine) modulate cellular response to growth, differentiation, and stress [17]. The cytosolic enzyme ornithine decarboxylase (ODC) catalyzes the decarboxylation of ornithine to putrescine which is the first and rate-limiting step of the polyamine biosynthetic pathway [9,17]. As polyamines are toxic to cells in excess, their pools are maintained rigidly in the normal resting cells by a stringent regulation of ODC at the transcriptional and translational level [21].

Recent studies showed an up regulation of ODC activity and putrescine accumulation following traumatic brain injury (TBI) [1,2]. However, it is not clear whether allosteric activation of the enzyme or de novo synthesis of enzyme protein is responsible for the increased catalytic activity observed after TBI. To assess the latter, ODC protein expression and enzyme activity was studied in the brains of rats sacrificed at 2 h, 6 h, 24 h, 72 h and 168 h after controlled cortical impact (CCI) brain injury and appropriate sham-operated controls.

All surgical procedures were conducted, and animals were cared for according to the animal welfare guidelines (*1985 Principles of the Guide for the Care and Use of Laboratory Animals, US Department of Health and Human Services Pub. 85-23*).

TBI was induced in adult Sprague–Dawley rats (300–350 g) under halothane anesthesia, with a CCI device as described earlier [1,2,6]. Briefly, brain injury was induced through a craniectomy (6 mm in diameter; between bregma and lambda and 1 mm lateral to midline) at a velocity of 3 m/s and 2 mm deformation. Sham-operated control rats were subjected to the same surgical procedure, including craniectomy, but received no cortical impact. The exposed cortex was covered with Surgicel and the wound was closed with sutures. Animals were allowed to recover from anesthesia, returned to their cages and allowed free access to standard laboratory rat chow and water. Animals were sacrificed at different periods (2 h, 6 h, 24 h, 72 h and 168 h) after the injury and the dissected ipsi- and contralateral cortices were snap frozen in liquid nitrogen and stored at −80°C.

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ODC immunoreactive protein levels were estimated by Western blot analysis. Briefly, frozen brain tissues were thawed and homogenized in 25 mM Tris–HCl (pH 7.4) buffer containing 2 mM EDTA and protease inhibitors (1 μg/ml aprotinin, 2 μM pepstatin-A, 2 μM leupeptin and 1 mM PMSF). The homogenate was centrifuged at 70 000 $\times g$ for 60 min at 4°C. Thirty micrograms protein equivalent of the supernatant (cytosol) was electrophoresed on a 10% polyacrylamide gel, transferred to a Hybond-ECL (Amersham) nitrocellulose membrane. The blot was washed twice (5 min each) in 25 mM Tris–HCl buffer (pH 7.4) containing 137 mM NaCl and 0.15% Tween 20 (TBS-T). After blocking for 60 min at room temperature in blocking buffer (TBS-T containing 2% (w/v) BSA), the blot was washed twice (5 min each) in TBS-T and incubated at room temperature with monoclonal anti-ODC antibody (Sigma Chemical) diluted to 1:5000 in blocking buffer. After washing (6×5 min) with TBS-T, the blot was incubated for 60 min at room temperature with HRP-coupled anti-mouse IgG (Transduction Laboratories) diluted to 1:5000 in blocking buffer. After washing (6×5 min) with TBS-T, the protein bands detected by the antibody were visualized using the ECL Western blotting kit (Amersham). The band intensities were quantitated by densitometric scanning. A linear signal ratio for quantitative densitometric analysis was determined by running a protein concentration curve. Before immunodetection, blots were stained with Ponceau-S to assess whether identical amounts of protein were aliquoted and to evaluate the protein transfer efficiency. The blots were subsequently stripped and reprobed with anti-β-tubulin antibody. The ODC densitometric data was normalized using β-tubulin immunoreactivity. ODC activity was assayed by determining the amount of $^{14}CO_2$ released from L-[1-^{14}C]ornithine at 37°C during a 1 h incubation period as described previously [1,2]. Protein was estimated by the method of Lowry et al. [14].

Monoclonal antibodies against ODC recognized a $\sim$55 kDa protein band. In the ipsilateral cortex of the injured rats, ODC protein levels were significantly increased at 2 h

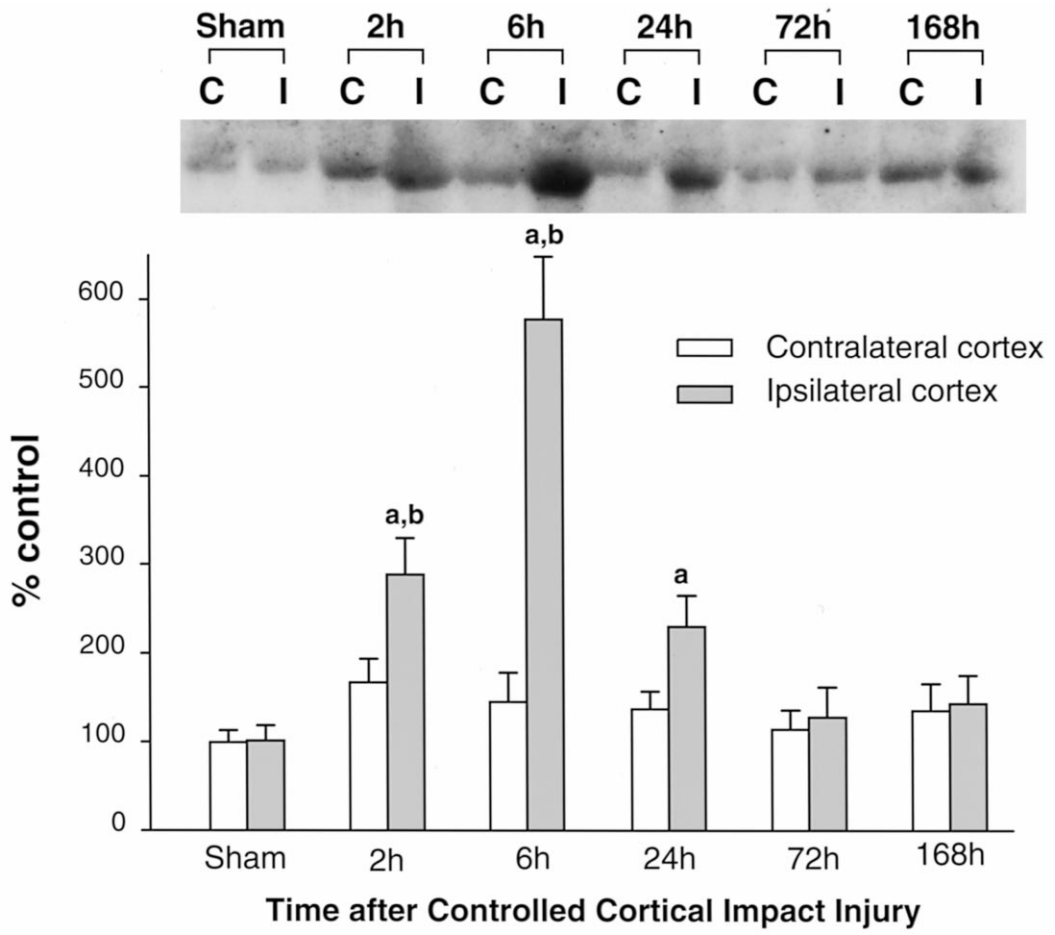

Fig. 1. Quantitative Western blot analysis of ODC protein levels in the ipsilateral (injured) and contralateral (uninjured) cortex from rats sacrificed at 2 h, 6 h, 24 h, 72 h and 168 h after CCI injury and sham-operated controls. Each value is a mean $\pm$ S.D. of five separate animals per group. Statistics: (a) $p < 0.05$ when compared with the corresponding contralateral cortex (ANOVA followed by Bonferroni-*t* test) and (b) $p < 0.05$ when compared with the sham-operated control rats sacrificed 6 h after the surgery (ANOVA followed by Dunnet's multiple comparisons test). Rats sacrificed at different periods after the sham-operation showed no significant differences between them. Inset: a representative Western blot.

(by ~3.5 fold, $p < 0.05$), 6 h (by ~5.5 fold, $p < 0.05$) and 24 h (by ~2.5 fold, $p < 0.05$) after the injury, when compared with either the sham-operated controls or the corresponding ipsilateral cortex of the injured animals (Fig. 1). By 72 h after the injury, ODC protein levels returned to basal control values. Compared with sham-operated controls, no significant changes were observed in the ODC protein levels in the contralateral cortex of the injured rats (Fig. 1). There were no statistically significant differences between the control rats sacrificed at different periods after the sham-operation. The levels of the cytoskeletal protein β-tubulin (used to normalize the ODC protein values) were not significantly altered at anytime after the injury (data not shown).

ODC activity was elevated significantly in the ipsilateral cortex of the TBI rats at 3 h (~13-fold, $p < 0.01$) and 6 h (by ~21-fold, $p < 0.01$) after the injury compared with either sham-operated controls or the corresponding contralateral cortex of the injured animals (Fig. 2). By 24 h after the injury, ODC activity returned to the normal control values (Fig. 2). ODC activities in the contralateral cortex of the injured animals were not significantly altered at anytime after the injury (Fig. 2).

Recent studies showed that TBI significantly elevates ODC catalytic activity [1,8]. The present study confirms and extends this observation. However, the mechanism of the induction of ODC activity following trauma is not clear. ODC activity may be stimulated due to an allosteric modulation of the existing enzyme activity or due to de novo synthesis of ODC protein. The present study shows that the trauma-induced increase in ODC activity is due to increased expression of ODC protein. As the half-life of the ODC protein is very short (in minutes) [5], the increased ODC protein level observed after TBI may be due to increased ODC gene expression and/or due to increased translation of ODC mRNA.

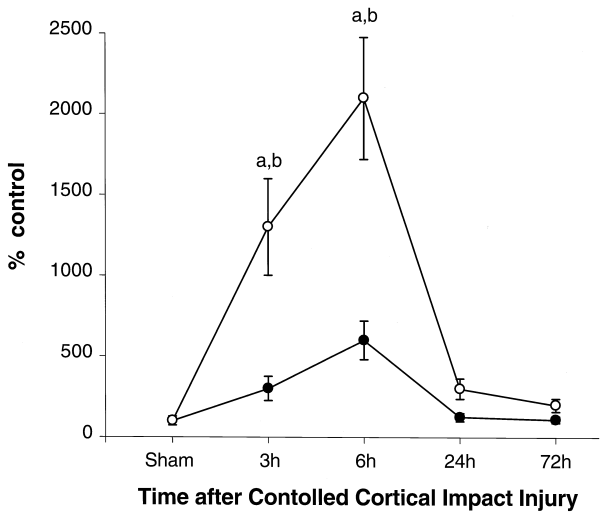

Fig. 2. ODC activity in the ipsilateral cortex (IC) and contralateral cortex (CC) at different periods after CCI injury. Open circles represent the ipsilateral cortex and closed circles represent the contralateral cortex. Values were given as percent of sham-operated control value (3.3 ± 0.9 pmol/mg protein h^{-1}; n of 15). There were no statistically significant differences between the sham-operated rats sacrificed at different periods. Each value is a mean ± S.E.M. of six animals per group. Statistics: (a) $p < 0.01$ when compared with the corresponding contralateral cortex (ANOVA followed by Bonferroni-t test) and (b) $p < 0.01$ when compared with the sham-operated control (ANOVA followed by Dunnet's multiple comparisons test).

TBI has been shown to be associated with blood-brain barrier (BBB) breakdown and vasogenic edema formation [1–3,12]. Elevated polyamine levels (a reflection of increased ODC protein expression and activity) observed after TBI [8,20] are thought to be playing an important role in BBB breakdown and vasogenic cerebral edema development [4,19]. Increased levels of polyamines mediate the increased cerebrovascular permeability [23]. Pretreatment of rats with the ODC inhibitor, difluoromethylornithine (DFMO) has been shown to decrease vasogenic edema and BBB breakdown after TBI [2,12]. DFMO pretreatment also decreases ischemia-induced edema and neuronal damage [11,18]. These studies suggest a role for the elevated ODC activity and concomitantly increased polyamine levels in mediating the BBB breakdown and the development of vasogenic edema after traumatic and ischemic brain injuries [3,23].

The pathophysiological mechanisms underlying neuronal dysfunction after ischemia and TBI are similar [16]. Though overall protein synthesis is markedly decreased after both TBI and ischemia [10,22], expression of certain proteins, such as the proto-oncogene c-*fos* and heat shock proteins hsp70 and heme oxygenase-1, is preferentially up regulated [7,13]. A transient early increase in ODC protein was shown in the vulnerable CA1 hippocampal region of ischemic gerbils, in which the overall protein synthesis was severely suppressed [4,15]. The present study showed that TBI also leads to a transient and significant increase in the ODC protein levels in the injured cortex. These results suggest that, after TBI, there is a preferential switch of synthesis to certain proteins instead of a complete breakdown of protein synthesis.

In conclusion, presently observed increased ODC protein levels suggest a significantly stimulated polyamine metabolism after TBI. This metabolic response occurring transiently between 2 and 24 h after the injury may be a promoter of BBB breakdown and vasogenic brain edema after TBI.

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