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The intricate involvement of the Insulin-like growth factor receptor signaling in mild traumatic brain injury in mice

Vardit Rubovitch [a], Shahaf Edut [a], Rive Sarfstein [b], Haim Werner [b], Chaim G. Pick [a,*]

[a] Dep. Anatomy and Anthropology, Sackler Faculty of Medicine, Tel Aviv University, Israel
[b] Dep. Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine, Tel Aviv University, Israel

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ABSTRACT

Insulin-like growth factor-1 (IGF-1) was suggested as a potential neuroprotective treatment for traumatic brain injury (TBI) induced damage (cognitive as well as cellular). The main goal of the present study was to evaluate the role of the IGF-1R activation in spatial memory outcome following mild traumatic brain injury. mTBI-induced phosphorylation of IGF-1R, AKT and ERK1/2, in mice hippocampus, which was inhibited when mice were pretreated with the selective IGF-1R inhibitor AG1024. IGF-1 administration prevented spatial memory deficits following mTBI. Surprisingly, blocking the IGF-1R signaling in mTBI mice did not augment the spatial memory deficit. In addition, this data imply an intriguing and complex role of the IGF-1 signaling axis in the cellular and behavioral events following mTBI.

Introduction

Traumatic brain injury (TBI) is one of the most common neurological disorders. The diagnosis of TBI includes a broad range of short- and long-term physical, cognitive, and emotional impairments, depending on the severity of the injury (Albensi, 2001; Waxweiler et al., 1995). In contrast to moderate and severe TBI in which brain morphological alteration is detectable (Graham et al., 2000), mild traumatic brain injury (mTBI) is commonly presented as a number of imprecise perceptual cognitive symptoms without diagnosable objective structural brain damage (the so-called "post-concussion syndrome") (Kibby and Long, 1996). We have previously reported that a non-invasive closed-head mTBI in mice induced short- and long-term deficits in a number of cognitive tests including impaired spatial memory and learning (Baratz et al., in press; Edut et al., 2008; Zohar et al., 2003). Nevertheless, no structural alterations were detected either by MRI or TTC staining (Tweedie et al., 2007; Zohar et al., 2003). Spatial memory deficits were also found in other mTBI animal models (Thompson et al., 2006; Wei et al., 2009).

Various cellular and biochemical changes in the brain, especially neuronal death (apoptosis, necrosis and gliosis), were found not only in moderate or severe TBI (Ferrer and Planas, 2003; Slemmer et al., 2008), but also in our mild TBI model (Tashlykov et al., 2007, 2009; Tweedie et al., 2007). mTBI-induced elevations in the levels of various pro-apoptotic proteins in different cortical and hippocampal regions of both injured and contralateral hemispheres (Tashlykov et al., 2007, 2009; Tweedie et al., 2007). However, additional, opposing data have been accumulating indicating a parallel, survival and anti-apoptotic pathway, pointing to the involvement of the serine–threonine kinase Akt/PKB (Noshita et al., 2002; Zhang et al., 2006). One of the major cascade that was found to activate Akt via phosphoinositide 3-kinase (PI3K) was the insulin-like growth factor-1 receptor (IGF-1R) (Povsic et al., 2003). Autophosphorylation of the IGF-IR also leads to the activation of the Ras— extracellular signal-regulated kinase (ERK) pathway (Franke, 2008; Vincent and Feldman, 2002). The serine–threonine protein kinase ERK plays distinct roles in the regulation of apoptosis in different cell types (Sawatzky et al., 2006). ERK1/2 have been implicated both as protective and damaging molecules signaling to define cell fate (Scheid et al., 1999).

The IGF-1 system is involved in growth, differentiation and survival signaling. [For a review see (Vincent and Feldman, 2002)]. It leads to the phosphorylation and inactivation of several pro- and anti-apoptotic proteins and transcription factors (Brami-Cherrier et al., 2002; Datta et al., 1997). Accumulating evidence indicates a major role for IGF-1 in central nervous system development and survival [for a review see (Aleman and Torres-Aleman, 2009)]. IGF-1 and its receptor are expressed in brain from the embryonic to the adult stage. Hence, IGF-1 has been implicated as a potential neuroprotective agent in brain injuries (Saatman et al., 1997) and in hypoxia–ischemia induced damage (Guan et al., 2003; Lin et al., 2009). In addition, the IGF-1R was found to be up-regulated following

* Corresponding author. Dept. of Anatomy and Anthropology, Sackler School of Medicine, Tel Aviv University, Tel Aviv, 69978, Israel. Fax: +972 6408287.
E-mail address: pickc@post.tau.ac.il (C.G. Pick).
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TBI (Madathil et al., 2009). In contrast to this protective role, reports have been published regarding a paradoxical effect of the IGF-1R signaling axis. Specifically, blockade of IGF-1 signaling has been reported to extend lifespan from invertebrates to mammals (Carter et al., 2002; Tang, 2006).

The main goal of the present study was to evaluate the involvement of the brain IGF-1R in minimal traumatic brain injury, and to assess its activation (phosphorylation) during this process. This study was aimed at revealing the unique and complex involvement of this pathway both at the biochemical and behavioral levels.

Materials and methods

Mice

Male ICR mice weighing 25–30 g were kept five per cage under a constant 12-h light/dark cycle, at room temperature (23 °C). Food (Purina rodent chow) and water were available *ad libitum*. Each mouse was used for one experiment and for one time point only. The Ethics Committee of the Sackler Faculty of Medicine approved the experimental protocol (M-04-063), in compliance with the guidelines for animal experimentation of the National Institutes of Health (DHEW publication 85–23, revised, 1995). The minimal possible number of animals was used and all efforts were made to minimize their suffering.

Brain injury

Experimental mTBI was induced using the concussive head trauma device described previously (Milman et al., 2005; Pan et al., 2003; Zohar et al., 2003). Briefly, mice were slightly anesthetized by inhalation of 0.5 ml of isoflurane in a closed glass chamber. Mice were placed under a device consisting of a metal tube (inner diameter 13 mm), placed vertically over their head. The animals were held in a way that the impact on the skull (closed scalp) was anteriolaterally, just anterior to the right ear. A sponge immobilization board was employed allowing small head movements during the injury, analogous to those that occur during closed-head injury in car accidents. The injury was induced by dropping a metal weight (30 g), from 80 cm height down the metal tube, striking the skull. Immediately after the injury, mice were placed back in their cages for recovery. Injured mice did not exhibit any apnea after the injury. The effect of the injury was studied at 7 and 30 days following the trauma, using different groups of mice at each time point (at least 10 mice per group).

Drug administration

IGF-1 (Cytolab, Rehovot, Israel) (4 μg/kg) was injected (i.p.) 24 and 48 h post injury. AG1024 (Calbiochem, Germany) was injected, i.p., (50 μg/kg) 1 h before the injury, and 24 and 48 h post TBI. These dosages and administration routes were chosen after reviewing the relevant literature and consulting with the developers of the drug (Drs. A. Gazit and A. Levitzki, Hebrew University, Jerusalem, Israel).

Western blots

Whole brains were removed 1, 24, and 72 h post mTBI, and hippocampi were immediately frozen in liquid nitrogen and homogenized with T-PER Tissue Protein extraction Reagent (Pierce, Rockford, IL), with appropriate protease inhibitors (Halt Protease Inhibitor Cocktail; Sigma-Aldrich). Samples were run in duplicate on precast 10% Bis-Tris gels (Bio-Rad) and transferred to nitrocellulose membranes. Blots were blocked for 1 h with Tris-buffered saline containing 0.01% Tween-20, 5% powered milk or 5% bovine albumin (Sigma-Aldrich). Membranes were incubated for 2 h at room temperature with antibodies against phospho-IGF-1R, phospho-Akt and phospho-ERK1/2 (Cell Signaling Technology) diluted 1:1000 and incubated with secondary horseradish peroxidase-linked antibodies (Jackson Immunoresearch, West Grove, PA) at room temperature for 1 h. Bands were visualized by enhanced chemiluminescence (Pierce Rockford, IL) and exposed to an X-ray film. Protein band intensities were quantified by using the TINA software. Uniform loading was verified by stripping and reprobing with antibodies against total IGF-1R, Akt or ERK1/2 (1:1000; Cell Signaling Technology).

Behavioral performance

Y-maze test

Spatial memory was assessed by using the Y-maze, which was first described by Dellu et al. (1992) and then subsequently validated as a task—requiring hippocampal function and spatial memory (Conrad et al., 1996). The Y-maze was constructed of black Plexiglas with three identical arms (30 × 8 × 15 cm). Overt cues were attached inside of the Y-maze. The test included two trials separated by two minute intervals. The first trial was 5 min with only two arms open (the start arm and the arm called "old" arm), and the third arm was blocked by a door (the novel arm). The mouse was put in the start arm in the part most distant from the other two arms. After the first (familiarization) run the mouse was put back into the cage for 2 min. The second run lasted 2 min, and all three arms were open. Time spent in each of the arms was measured. Between each run and between each mouse the maze was cleaned with 70% ethanol. The new arm preference index was calculated as follows: (time in the new arm − time in the old arm) / (time in the new arm + time in the old arm).

Data analysis

All results are given as mean ± SEM and data were analyzed using one way ANOVA; statistical significance was set at $^{*}p$, ^{y}p, $^{z}p<0.05$; $^{**}p$, ^{yy}p, $^{zz}p \ll 0.01$; $^{***}p$, ^{yyy}p, $^{zzz}p \ll 0.001$. p values of *post hoc* tests were adjusted using the Fisher LSD test and a nominal significance level of 0.05 was used.

Results

The main goal of our study was to evaluate the IGF-1R pathways activated following minimal traumatic brain injury. The rationale for our focus on the IGF-1R pathway was its confirmed pro-survival and anti-apoptotic activities, and the fact that the IGF-1R axis is involved in a number of neuronal processes.

mTBI-induced IGF-1R phosphorylation

Initially we examined the effect of mTBI on IGF-1R phosphorylation. For this purpose, mice were subjected to mTBI, and brains were removed after 1, 24 and 72 h. Hippocampi extracts prepared from brain tissues were subjected to immunoblot analyses. Results indicated that mTBI induced a significant elevation in IGF-1R phosphorylation 24 h post mTBI (1.87 ± 0.18 fold over control for the right, injured hippocampus; $n = 5–7$, $p<0.0001$; Fig. 1A). Similar changes were found in the left hippocampus (data not shown).

mTBI-induced Akt phosphorylation

Since the PI3-kinase/AKT is one of the main downstream pathways activated by the IGF-1R, we next tested whether Akt was also activated in response to mTBI. As shown in Fig. 2A, mTBI induced a significant phosphorylation of hippocampal Akt in injured mice as compared to control mice. However, while the major phosphorylation of the IGF-1R was found 24 h post injury, significant Akt phosphorylation was found already after 1 h, and remained constant up to 72 h