Traumatic Brain Injury Causes Long-Term Reduction in Serum Growth Hormone and Persistent Astrocytosis in the Cortico-Hypothalamo-Pituitary Axis of Adult Male Rats

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Abstract

In humans, traumatic brain injury (TBI) causes pathological changes in the hypothalamus (HT) and the pituitary. One consequence of TBI is hypopituitarism, with deficiency of single or multiple hormones of the anterior pituitary (AP), including growth hormone (GH). At present no animal model of TBI with ensuing hypopituitarism has been demonstrated. The main objective of this study was to investigate whether cortical contusion injury (CCI) could induce long-term reduction of serum GH in rats. We also tested the hypothesis that TBI to the medial frontal cortex (MFC) would induce inflammatory changes in the HT and AP. *Methods:* Nine young adult male rats were given sham surgery ($n = 4$) or controlled impact contusions ($n = 5$) of the MFC. Two months post-injury they were killed, trunk blood collected and their brains and AP harvested. GH was measured in serum and AP using ELISA and Western blot respectively. Interleukin-1 β (IL-1 β) and glial fibrillary acidic protein (GFAP) were measured in the cortex (Cx), HT, and AP by Western blot. *Results:* Lesion rats had significantly ($p < 0.05$) lower levels of GH in the AP and serum, unaltered serum IGF-1, and significantly ($p < 0.05$) higher levels of IL-1 β in the Cx and HT and GFAP in the Cx, HT, and AP compared to that of shams. *Conclusion:* CCI leads to a long-term depletion of serum GH in male rats. This chronic change in GH post-TBI is probably the result of systemic and persistent inflammatory changes observed at the level of HT and AP, the mechanism of which is not yet known.

Key words: anterior pituitary; growth hormone; hypopituitarism; hypothalamus; TBI

Introduction

TRAUMATIC BRAIN INJURY (TBI) is a leading cause of mortality and long-term disability in the United States and throughout the world. In addition to the many acute focalized and systemic changes caused by the initial injury, TBI-induced long-term consequences can include more global alterations in the endocrine system as a result of the organism's systemic response to the injury at the level of the hypothalamus (HT) and the pituitary gland (Powner et al., 2006). The many reviews on the impact of TBI on the neuroendocrine system cite evidence for suppression of the stress, growth, and reproductive axes due to hypopituitarism with single or multiple hormonal deficiencies. These include hyposecretion of corticosterone (CORT) (probably due to altered corticotropin releasing hormone [CRH]), adrenocorticotrophic hormone (ACTH), growth hormone (GH) (probably due to altered growth hormone releasing hormone [GHRH]/somatostatin system), and luteinizing hormone (LH) (probably due to

altered luteinizing hormone releasing hormone [LHRH] system) (Behan et al., 2008). Other hormonal axes affected include the thyroid (thyrotropin releasing hormone [TRH]/thyroid stimulating hormone [TSH]/tri-iodothyronine (T3)/tetra-iodothyronine [T4]) axis (Schneider et al., 2006) and the vasopressin system (Agha et al., 2004b; Powner et al., 2006). However, the mechanisms causing long-term suppression of the hypothalamic-pituitary (HP) axis after brain injury are not completely known.

One mechanism by which cortical brain injury might induce pathological changes in structures distal to the cortical injury, like the HP and the AP (and their functions), could be due to the persistence (Nonaka et al., 1999) and spread of inflammatory factors at the site of injury, which in turn result in secondary necrosis and apoptosis of distal brain tissue. Previous studies have shown that TBI can induce diffuse and long-term degeneration of subcortical tissues. For instance, Holmin and Mathiesen demonstrated the persistence of inflammation and astrocytosis at the site of injury (cortex [Cx])

at 3 months post-TBI (Holmin and Mathiesen, 1999), and Bramlett et al. presented evidence of inflammatory and atrophic changes in the cortical, hippocampal, and thalamic regions at 8 weeks to 1 year post-injury (Bramlett et al., 1997; Bramlett and Dietrich, 2002). Smith et al. found signs of inflammation and progression of tissue atrophy up to 1 year post-injury, leading to degenerative changes in cortical and hippocampal areas. The authors concluded that "the rate of atrophic changes did not diminish and may have been accelerating even at 1 year post-injury, supporting the possibility that active degenerative processes were initiated" (Smith et al., 1997).

It is also possible that proinflammatory factors produced at the injury site might affect distant structures by means of volume transmission (VT), as proposed by Bach-y-Rita (2003), such that toxic factors secreted by injured cells may act not only on the respective post-synaptic neurons (within the synaptic regions), but may diffuse through the extracellular space or the cerebral ventricles to cause more systemic inflammation and neuronal loss. We hypothesize that the persistence of proinflammatory cytokines produced and secreted at the site of cortical injury can lead to the induction of inflammatory reaction in the AP and HT, and may therefore affect the functions of the endocrine system. Other signaling mechanisms could include trans-synaptic (Koliatsos et al., 2004) as well as anterograde and retrograde neuronal degeneration from the site of injury to the more distal sites (Sorensen et al., 1996; Bechmann and Nitsch, 1997), also eventually affecting some of the endocrine functions. While we know something about the effects of TBI on endocrine function based on human studies (Ceballos, 1966; Agha et al., 2004a, 2004b, 2005a, 2005b; Aimaretti et al., 2004, 2005b; Bondanelli et al., 2004; Herrmann et al., 2006; Powner et al., 2006; Schneider et al., 2006; Tanriverdi et al., 2006), there is still some uncertainty about the mechanisms by which long-term alterations in the endocrine system (like suppression of serum GH in TBI subjects) evolve over time, and whether such changes could be prevented by therapeutic interventions.

One of the hormonal systems of interest during the aftermath of TBI is the GH/IGF-1 system, because of the development of GH deficiency during chronic stages post-TBI (indicating the possibility of a gradual evolution of the symptoms post-injury). Also, GH and IGF-1 play a role in myelin formation (Carson et al., 1993), neuronal plasticity (Aberg et al., 2006), and vascular tone (Sonntag et al., 1997), factors that are essential for brain repair after an injury/trauma. Therefore, in this study we looked at the long-term effects of TBI on the serum GH profile and inflammatory markers in the HP axis at 2 months post-injury, since persistence and progression of inflammatory and atrophic changes previously reported (Bramlett et al., 1997; Smith et al., 1997; Pierce et al., 1998; Holmin and Mathiesen, 1999) were evident one month post-injury (Smith et al., 1997; Pierce et al., 1998). We also measured serum levels of IGF-1, because it is considered a biomarker for serum GH levels (Blum et al., 1993; de Boer et al., 1995; Jones and Clemmons, 1995) and because its production in the liver is dependent on serum GH. Changes in hormonal profile or markers of inflammation during the acute phase post-injury, directly due to the injury and stress, cannot be assumed to be either transient or permanent. In the present study, the 2-month recovery time enabled us to observe whether changes persisted. We tested the hypothesis that TBI

to the frontal Cx of adult male rats will result in persistent reductions in GH levels in the serum and/or AP at 2 months post-TBI. The results confirmed this hypothesis. We also tested whether this injury would result in enhanced expression of the markers of inflammation, like IL-1 β and GFAP (a marker of astrogliosis) that persist in the HP axis at 2 months post-TBI. Our results strongly support the role of inflammation in the suppression of GH during the chronic phase of severe cortical brain injury.

Methods

Animals

Nine young adult male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA), 3 months of age, were maintained on a 12:12 h (light/dark) reverse lighting schedule, with lights on at 9:00 P.M. The animals were housed in a clean, temperature- ($22 \pm 1^\circ\text{C}$) and humidity-controlled facility approved by the American Association for the Accreditation of Laboratory Animal Care in accordance with NIH guidelines, with food and water provided *ad libitum* both before and after surgery. The Emory University Institutional Animal Care and Use Committee (IACUC) approved all the animal care and experiment protocols (protocol no. 146-2005).

Experimental design

The rats (Ss) were allowed to acclimatize to their quarters for 2 weeks, handled for another 2 weeks, and then subjected to TBI or sham surgeries under isoflurane and nitrous oxide anesthesia as described below. The Ss were then allowed to recover for 2 months, during which time they were housed individually, with food and water *ad libitum*, and handled once a week when they were transferred to clean cages with fresh food and water. Two months post-TBI, the Ss were subjected to 5% isoflurane anesthesia for 3 min and decapitated. Trunk blood was collected and allowed to stand at room temperature for 30 min, followed by centrifugation at 14,000 rpm for 10 min, when the serum was separated and stored at -80°C until the time of hormone assay. The brain was quickly extracted and frozen on dry ice after dissection of the stalk median eminence (SME), which was stored frozen at -80°C . The HT was dissected with the posterior part of the optic chiasm as the anterior boundary, the anterior part of the mammillary body as the posterior limit, and the lateral hypothalamic sulci as the lateral limits (Francis et al., 2004). The AP was isolated from the base of the cranial cavity after removing the neural lobe and frozen on dry ice. Samples were stored at -80°C until the time of hormone assay.

Surgeries

Rats were subjected to sham surgeries ($n = 4$) or TBI ($n = 5$) according to procedures described elsewhere (Cutler et al., 2007). Briefly, the rats were initially anesthetized under 5% isoflurane anesthesia for 3 min, 45 s, and nitrous oxide (700 mmHg/min) with oxygen (400 mmHg/min) as a carrier. The head was then shaved and the animal placed into a Kopf stereotaxic device with a thermal blanket and maintained under anesthesia (isoflurane 2.5%) for the rest of the surgery. Heart rate and blood oxygen levels were maintained above 300/min and 90 mmHg, respectively, using a blood oximeter. The entire surgery lasted 25–30 min, during which time the

core body temperature was monitored using a rectal temperature probe. There was no significant difference in body temperature between the two groups during this time period (data not shown). The scalp was cleaned with betadine and isopropyl alcohol, and a mid-line incision was made. Bilateral craniotomy was performed using a trepan drill (6 mm diameter) at 3 mm rostral to bregma with the caudal border touching the bregma. The animals were subjected to bilateral controlled cortical impact (CCI) injury 2.5 mm from the brain surface using a pneumatic device (5 mm diameter) to the MFC, at a velocity of 2.5 m/s with a dwell time of 500 ms. The scalp was sutured after bleeding was controlled, and the animals were permitted to recover on a warm blanket for 1 h before being returned to their home cages. Sham surgery consisted of the procedures for TBI surgery, including exposing the skull and taking the measures for craniotomy, but without the craniotomy. The animals were then maintained under anesthesia for approximately the same duration as the TBI subjects. Procedures for recovery after surgery were as described above. Neither the shams nor the lesion animals received special handling of any sort. Body weights were recorded before surgery and at the time of decapitation. No other parameters or activities were monitored. Brains and serum were collected as described above.

Materials

The following were purchased and employed: tissue protein extraction reagent (T-PER, 78510) and SuperSignal West Dura Extended Duration Substrate (34076, Thermo Scientific, Rockford, IL); milk diluent/blocking solution concentrate (50-82-00, KPL, Gaithersburg, MD); Immobilon transfer membranes (IPVH00010, Millipore, Bedford, MA); 4-20% Tris-HCl Criterion Precast Gel (345-0033, Bio-Rad, Hercules, CA); goat anti-rat GH (AF1566, R&D Systems, Minneapolis, MN); rabbit polyclonal to rat IL-1 β (ab9787-100, Abcam, Cambridge, MA); rabbit anti-rat GFAP (AB5804, Chemicon, Temecula, CA); mouse monoclonal to β -actin (ab8226-100, Abcam); GH ELISA kit (Linco Research, St. Charles, MO); IGF-1 ELISA kit (Immunodiagnostic Systems, Fountain Hills, AZ); goat anti-mouse IgG (04-18-18, KPL); goat anti-rabbit IgG (074-1506, KPL); rabbit anti-goat IgG (14-13-06, KPL); and protease inhibitor cocktail (Sigma Aldrich, St. Louis, MO).

Tissue processing

All the collected tissue (Cx, HT, and AP) was homogenized in T-PER containing protease inhibitor cocktail, spun at 14,000 rpm for 10 min at 4°C, and the supernatant collected and used for sample preparation for Western blots. Briefly, the protein concentration in the supernatant was measured using Coomassie blue reagent, and the Cx, HT, and AP (not SME) homogenates were used to prepare the samples for SDS-PAGE, as described elsewhere (Cutler et al., 2007), to a final concentration of 1.2 μ g/ μ L. AP Western samples with a concentration of 0.12 μ g/ μ L were used for the assay of GH.

Hormone assays

Both the GH and IGF-1 were assayed in serum samples using sandwich ELISA. Samples were run in duplicates along with the standards, and quality controls were provided with the respective kit. The protocol was followed as recommended

by the kit manual (for GH and IGF-1). The sensitivity of the GH and IGF-1 assays were 0.07 ng/mL and 63 ng/mL, respectively. The standard curve was plotted on a semi-log graph. The concentration of the samples was interpolated from the curve. The values of serum GH and IGF-1 are expressed in ng/mL.

Protein electrophoresis and Western blot analysis

The samples for the SDS-PAGE were prepared as described above, then loaded onto 4–20% gradient gels (12 μ g/well for IL-1 β , 6 μ g/well for GFAP, and 0.6 μ g/well for GH) and subjected to electrophoresis at 200 V for 55 min. The gels were blotted onto nitrocellulose membranes at 100 V for 30 min. The blots were treated with blocking solution overnight and then probed for IL-1 β , GFAP, GH, or β -actin using the respective primary antibodies followed by appropriate HRP-conjugated secondary antibody. The blots probed for IL-1 β and GFAP were incubated overnight at 4°C with the primary antibody, while the blots probed for GH and β -actin were incubated for 2 h at 4°C. The antibody dilutions used for probing IL-1 β , GFAP, GH, and β -actin were 1:1000, 1:2000, 1:2000, and 1:5000, respectively, for the primaries, and 1:2000 for all the secondaries. Blots were developed using Pierce chemiluminescent reagents and scanned using a Kodak digital system. Only blots with band intensities in the linear range (not saturated) were used for quantification. Bands were quantified using densitometry.

Statistical analysis

The GH assay standard curve was generated with Graph-Pad Software (La Jolla, CA) using non-linear regression analysis. For IGF-1 the standard plot was made on semi-log graph and the values interpolated. For the final statistical analysis of GH, IGF-1, IL-1 β , and GFAP, Student's *t* test (one-tailed) was used to compare the data between the sham and lesion groups at a significance level set to $p < 0.05$. If the variance between the two groups was significantly different, we then used the Welsch correction (*t* test for unequal variance) for the analysis.

Results

TBI causes a reduction in serum GH

We tested the hypothesis that TBI to the frontal Cx will lead to chronic reduction in serum GH. We observed a significant reduction in the levels of serum GH (mean $\pm$ SEM ng/mL) at 2 months post-injury (7.368 ± 3.236) compared to that of sham-operated animals (29.25 ± 8.459 , one-tailed, $t = 2.641$, $df = 7$, $p \leq 0.0167$). These findings suggest that cortical TBI has a long-term suppressive effect on serum GH levels (Fig. 1) and leads to alterations in the AP somatotrophic system.

TBI induces reduction in AP-GH at 2 months

An analysis of the effect of TBI on GH content in the AP (mean $\pm$ SEM arbitrary units, using Western blots, β -actin corrected; Fig. 2A) also revealed significant suppression ($p \leq 0.0360$) in the expression of the 22 kDa isoform of GH in the TBI group (9.411 ± 1.485) compared to that of shams (13.6 ± 1.183 , one-tailed, $t = 2.117$, $df = 7$) (Fig. 2B). We did not find any

FIG. 1. TBI results in significant ($p \leq 0.0167$) reduction in serum GH levels at 2 months post-injury. Data shown here represent the mean $\pm$ SEM. The number of animals/group is indicated in the bar.

significant difference in the 20 kDa isoform of GH between the two groups (one-tailed, $t = 0.4000$, $df = 7$, for 20 kDa GH) (Fig. 2C).

TBI did not alter serum IGF-1 levels

Since TBI resulted in a decline in GH levels in the serum, we measured the serum IGF-1 level, which is widely used as a marker of somatotrophic activity and whose expression from the liver is dictated by the levels of GH in the serum. There was no significant difference ($p \geq 0.0641$) in the level of IGF-1 between the two groups as analyzed by one-tailed Student's t test ($t = 1.724$, $df = 7$) at $p < 0.05$ (Fig. 3).

Evidence of IL-1 β production in the Cx and HT after TBI

Previous studies have shown evidence of TBI-induced persistent inflammation in the Cx at 3 months post-injury (Holmin and Mathiesen, 1999). Our study assayed the Cx, HT, and AP at 2 months post-injury for persistence of inflammation (using IL-1 β as the marker) and a decline in GH content in the AP. We specifically measured both the unglycosylated (17 kDa) and the glycosylated (25 kDa) isoforms of IL-1 β , as both forms are known to be secreted in equimolar amounts by the cells, and glycosylated IL-1 β has a weak activity (Livi et al., 1991). It appears that glycosylated IL-1 β is not the precursor for the 17 kDa form, and glycosylation reaction appears to be independent and a random event. We found significant ($p \leq 0.0094$) upregulation of IL-1 β protein (17 kDa) expression in the cortical contusion region in the TBI Ss (one-tailed, $t = 3.042$, $df = 7$; Fig. 4A) compared to that of sham-operated Ss. The TBI Ss also had significantly ($p \leq 0.0146$ for 17 kDa and $p \leq 0.0094$ for 25 kDa) higher levels of IL-1 β (both 17 kDa and 25 kDa) in the hypothalamus (Fig. 5A and B) compared to that of sham Ss at 2 months post-injury (one-tailed, $t = 3.026$, $df = 5$ for 17 kDa; one-tailed, $t = 3.044$, $df = 7$ for 25 kDa). However, TBI did not cause any alterations in the levels of either 17 kDa or 25 kDa IL-1 β in the AP (Fig. 6A and B) at 2 months post-injury or in the levels of 25 kDa IL-1 β in the Cx (Fig. 4B; $p \geq 0.1196$) compared to sham at 2 months post-injury.

FIG. 2. GH content (β -actin corrected) in the AP. (A) Blot picture of the two isoforms of GH (arrow A, 22 kDa; arrow B, 20 kDa) from AP. Lanes 1–4 indicate sham subjects; lanes 5–9 indicate lesion subjects. (B) Significant ($p \leq 0.0360$) reduction in the amount of 22 kDa GH isoform in the lesion animals compared to that of sham-operated controls. (C) Content of the 20 kDa GH isoform in the two groups. TBI did not have any significant effect on the 20 kDa isoform of GH ($p \geq 0.3505$). The number of animals/group is indicated in the bar of each graph. Data shown here represent mean $\pm$ SEM of the band intensity.

Evidence of astrogliosis in the Cx, HT, and AP at 2 months post-TBI

Since measures of IL-1 β suggested inflammation after TBI, we asked whether this could be the result of post-injury glial activity. Induction of GFAP (a marker for the proliferation/expansion of glial cells) is considered evidence of injury-induced astrogliosis (Vitellaro-Zuccarello et al., 2008). We therefore measured the expression of GFAP in the Cx, HT, and AP to determine whether astrogliosis persists in the injured Ss at 2 months post-TBI. We found significant ($p \leq 0.0038$, 0.0125 and 0.0165 for Cx, HT, and AP, respectively) upregulation of GFAP protein expression in the Cx (one-tailed, $t = 4.966$, $df = 4$; Fig. 7); HT (one-tailed, $t = 2.841$, $df = 7$; Fig. 8); and AP (one-tailed, $t = 2.759$, $df = 6$; Fig. 9) of TBI Ss compared to that of sham-operated animals.

FIG. 3. Unaltered serum IGF-1 in the face of reduced serum GH. TBI-induced GH reduction at 2 months post-injury was not accompanied by reduction in serum IGF-1 levels. Data shown here represent the mean $\pm$ SEM.

Discussion

In this study we demonstrated that bilateral contusions of the MFC in young adult male rats leads to the suppression of GH in serum and AP at 2 months post-injury. Furthermore, this chronic change is accompanied by a similarly chronic inflammatory reaction in the HP axis involving upregulation of IL-1 β and GFAP protein levels and upregulation of GFAP and IL-1 β in the Cx. To our knowledge, this is the first study to show alterations in GH levels and persistence of inflammatory reaction in the HP axis 2 months after injury in an animal model of cortical TBI. Similar studies demonstrating long-lasting changes include one by Holmin and Mathiesen (1999), who showed persistent inflammation at the site of injury in the Cx at 3 months post-injury, and others showing progressive demyelination and atrophic loss of neuronal elements in subcortical brain structures from 8 weeks to 1 year post-TBI (Bramlett et al., 1997; Smith et al., 1997; Bramlett and Dietrich, 2002, 2004, 2007; Rodriguez-Paez et al., 2005). Taken together, these observations suggest that cortical TBI inflicts long-lasting destructive changes in brain tissue that is remote from the site of the initial CCI. Specifically, our findings and others in humans with TBI (Kelly et al., 2000; Lieberman et al., 2001; Agha et al., 2004a; Aimaretti et al., 2004; Bondanelli et al., 2004) demonstrate that cortical TBI can have substantial effects on the expression of the GH system, supporting clinical studies and replicating the results in an animal model of CNS injury.

Implications of GH/IGF-1 deficiency in TBI subjects

GH/IGF-1 deficiency has been shown to affect cognitive function. For example, Leon-Carrion et al. (2007) showed a GH-related cognitive impairment in patients who develop a GH deficiency post-TBI. These patients also had deficits in memory and attention. Similar cognitive deficits (Sartorio et al., 1995, 1996; Deijen et al., 1996, 1998) responsive to GH treatment (Papadakis et al., 1995; Sartorio et al., 1995; Deijen et al., 1998; Soares et al., 1999) were demonstrated in adults with GH deficiency who were *not* victims of TBI, implicating GH (and the impact of GH deficiency) in the functioning of memory and cognition. It is interesting to note that adult-onset GH deficiency in young adults is related to poor sur-

FIG. 4. (A) TBI induces significant ($p \leq 0.0094$) expression of unglycosylated IL-1 β (17 kDa) protein in the cortical contusion area compared to that of sham-operated animals, supporting the idea of persistent production of proinflammatory cytokines at the site of injury even at 2 months post-injury. (B) No significant ($p \geq 0.1196$) change in the level of 25 kDa glycosylated IL-1 β was observed between the two groups at 2 months post-injury in the cortical (contused) area. Data shown here represent mean $\pm$ SEM of the ratio of IL-1 β to β -actin from the same samples. The number of animals/group is indicated in the bar.

vival of new neurons in the dentate gyrus (Lichtenwalner et al., 2006), severe impairment of brain glucose utilization, and significant decline in hippocampal ATP content (Sonntag et al., 2006). These studies and reviews provide support for the role of GH/IGF-1 in cognitive function in adult-onset GH deficiency/aging. Given the apparent role of GH and IGF-1 in neuronal plasticity (Aberg et al., 2006), memory, and cognitive performance (Deijen et al., 1996, 1998; Sonntag et al., 2005), it is likely that a decline in serum GH levels post-TBI may contribute to long-term deficits in memory functions and neuronal/cellular loss in the brain, which can be exacerbated and persistent long after traumatic injuries to the cerebral Cx.

Causes of GH deficiency

Hypopituitarism due to TBI could be caused by several factors, one of which is obviously direct injury to the AP or the

FIG. 5. Persistent induction of IL-1 β in the hypothalamus of lesion animals compared to that of sham-operated animals at 2 months post-injury. **(A)** Significant ($p \leq 0.0146$) upregulation of unglycosylated (17 kDa) IL-1 β . **(B)** Significant ($p \leq 0.0094$) upregulation of glycosylated (25 kDa) IL-1 β in the lesion animals compared to that of shams. The β -actin-corrected data are represented as mean $\pm$ SEM in both A and B. The number of animals/group is shown in the bar.

FIG. 6. IL-1 β levels (both unglycosylated **(A)** and glycosylated **(B)** forms) in the AP of sham and lesion animals at 2 months post-surgery. The β -actin-corrected data are represented as mean $\pm$ SEM for both **(A)** and **(B)**. The number of animals/group is shown in the bar.

HT, but this was not the case in our study, because the injury was inflicted bilaterally in the dorsal MFC, not proximal to the HT or AP. This does not rule out the possibility of secondary seepage bleeding in cortical injuries, ventriculomegaly, or mechanical forces (compression and diffuse axonal injury caused by the impact), which then could induce chronic damage to the HP/BS region. Among the secondary effects of impact injury are edema formation (Roof et al., 1993), increased intracranial pressure (ICP) (Kita and Marmarou, 1994), and hemorrhage (Powner et al., 2006) in the vasculature of the HP and/or AP. It is important to note also that increased ICP is assumed to contribute to anterior lobe infarction (in the AP) through interruption of the hypothalamohypophyseal portal blood supply (Ceballos, 1966; Harper et al., 1986). Acute studies in female rats in our laboratory revealed diffuse hematoma in the HT and BS (Kasturi and Stein, unpublished report). We also found development of edema at 48 h post-TBI in subcortical structures including the thalamus, HT, AP, and BS, in both young and aged ovariectomized females (Kasturi et al., 2008). Based on these and other studies demonstrating long-term subcortical neuronal degeneration and persistence

of inflammation, we suggest that lesion/injury at the level of the HP axis with or without accompanying pituitary dysfunction might play a role in the development of GH hyposecretion after TBI, with a probable alteration in the levels or activity of the GHRH and somatostatin systems in the HT.

Other factors that could have contributed to HP failure in this study include inflammatory mediators like IL-1 and TNF, which are released as a result of inflammation at the primary injury site (Cx) (He et al., 2004) and HP axis. Others have shown that TBI to the Cx caused persistent inflammation at the injury site at 3 months after the initial impact (Holmin and Mathiesen, 1999). In our experiment with bilateral damage to the frontal Cx, we found a significant increase in the expression of IL-1 β and increased expression of GFAP in the HP axis at 2 months post-injury. We speculate that inflammatory factors produced in the cortex could diffuse to distant sites by means of VT (Bach-y-Rita, 2003), either through the ventricles or by movement through extracellular fluid and spaces, thereby activating further cytokine (IL-1) production downstream from the initial injury (Warner et al., 1987) and activating a rolling cascade of inflammatory reaction.

FIG. 7. TBI induced a significant ($p \leq 0.0038$) upregulation of GFAP expression in the cortical contusion area compared to that of sham-operated animals at 2 months post-injury, suggesting persistence of gliosis. Data shown here represent mean $\pm$ SEM of the ratio of GFAP to β -actin from the same samples. The number of animals/group is indicated in the bar.

The results of our study demonstrating increased upregulation of GFAP and IL- β and persistently decreased secretion of GH support the idea of persistent inflammation and astrogliosis at the level of the HP axis even at 2 months post-TBI. However, the actions of the inflammatory factors in both the acute inflammatory response (Patel et al., 2003) and in the reparative process during the chronic phase post-injury (Lenzlinger et al., 2001; Morganti-Kossmann et al., 2002), and the role of IL-1 β in neuroprotection/growth (Horie et al., 1997; Lu et al., 1997), offer a possible explanation for IL-1 upregulation in neurorepair in the HP axis. Future studies are needed to validate these interesting but admittedly speculative ideas. The delayed and persistent upregulation of astrocytes at the

FIG. 8. TBI-induced significant ($p \leq 0.0125$) upregulation of GFAP expression in the HT compared to that of sham-operated animals at 2 months post-injury, suggesting persistence of gliosis-like changes at a site remote from the injury area (MFC). Data shown here represent the mean $\pm$ SEM of the ratio of GFAP to β -actin from the same samples. The number of animals/group is shown in the bar.

FIG. 9. TBI-induced significant ($p \leq 0.0165$) upregulation of GFAP expression in the AP compared to sham surgery at 2 months post-injury, suggesting long-term gliotic changes in tissue more distal from the site (MFC) of injury. This may indicate chronic cellular/molecular alterations in the HP axis as a result of MFC injury. Data shown here represent mean $\pm$ SEM of the ratio of GFAP to β -actin from the same samples. The number of animals/group is shown in the bar.

site of injury, along with the increased expression of IL-1 β in the same region, is consistent with the previous findings (Holmin and Mathiesen, 1999; Pearson et al., 1999; Raghavendra Rao et al., 2000), demonstrating the possible involvement of astrocytes during the chronic recovery stage. The biological significance of glycosylation of IL-1 β is not clear. However, we do know that the glycosylated isoform is secreted in almost equimolar amounts from the cells along with the mature (unglycosylated) form and that it possesses about 20% of the activity of the mature form (Livi et al., 1991). Future studies need to address the significance of glycosylation in the context of inflammation and repair.

The unaltered serum IGF-1 levels in the face of reduced serum GH as a result of TBI measured at 2 months post-injury were unexpected, since GH regulates liver IGF-1 production, and IGF-1 is considered a reliable marker of GH status (Blum et al., 1993; de Boer et al., 1995; Jones and Clemmons, 1995). However, the reliability of serum IGF-1 as an indicator of GH deficiency is being debated (Aimaretti et al., 1998, 2003; Granada et al., 2000). Specifically, the consensus guidelines for the diagnosis and treatment of adults with GH deficiency conclude that, "a normal IGF-1 does not rule out GH deficiency" (Ho, 2007). The guidelines also note that, "as the GH axis may recover after TBI, testing for GHD should be undertaken no sooner than 12 months after the injury." In line with this view, it is likely that in our model of TBI, at 2 months post-injury the rats may be in a state of recovery with respect to the GH-IGF-1 axis, or may undergo further degenerative changes in the GH system similar to that observed in neuronal systems in other TBI studies (Bramlett et al., 1997; Smith et al., 1997; Holmin and Mathiesen, 1999; Bramlett and Dietrich, 2002, 2004, 2007; Rodriguez-Paez et al., 2005). Therefore, further long-term studies are needed to monitor whether TBI-induced reduction in serum GH as found in this study is a transient or a permanent phenomenon. Also, functional pituitary tests like the combined arginine + GHRH test (Gasco

et al., 2008) may be considered in evaluating GH deficiency and pituitary dysfunction. We also do not know whether these rats have isolated GH deficiency or multiple deficiencies of pituitary hormones.

Conclusion

In summary, bilateral contusions to the dorsal MFC chronically suppress GH levels in the serum and AP, and induce IL-1 β and GFAP in the Cx and HP axis when measured up to 2 months after the initial injury. It is not known at present whether the long-lasting changes we observed in GH, IL-1 β and GFAP could be upstream precursors of other pathological lesions observed post-TBI—e.g., the deposition of beta-amyloid and/or amyloid precursor proteins in the cortical regions or the neuronal loss reported in an experimental model of TBI (Lewen et al., 1995; Pierce et al., 1996; Iwata et al., 2002). It is also possible that in pediatric TBI, symptoms and deficits are exacerbated by GH deficiency occurring weeks or months after the initial insult, as happens in aged subjects (Aimaretti et al., 2005a; McDonald et al., 2008). Further studies are also needed to examine the effects of immunosuppressive drugs such as Cyclosporin A (Mirzayan et al., 2008) or progesterone (Vanlandingham et al., 2008) on the etiology of GH deficiency in animal TBI. Furthermore, it is important to recognize that there are persistent systemic changes in response to “focalized” injury, and these enduring changes may play a critical role in the extent to which neuronal repair and restoration of function can occur.

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Author Disclosure Statement

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