Published in final edited form as:
Radiat Res. 2014 January ; 181(1): 21–32. doi:10.1667/RR13475.1.

Differential Expression of Homer1a in the Hippocampus and Cortex Likely Plays a Role in Radiation-Induced Brain Injury

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Abstract

Fractionated partial or whole-brain irradiation is the primary treatment for metastatic brain tumors. Despite reducing tumor burden and increasing lifespan, progressive, irreversible cognitive impairment occurs in >50% of the patients who survive >6 months after fractionated whole-brain irradiation. The exact mechanism(s) responsible for this radiation-induced brain injury are unknown; however, preclinical studies suggest that radiation modulates the extracellular receptor kinase signaling pathway, which is associated with cognitive impairment in many neurological diseases. In the study reported here, we demonstrated that the extracellular receptor kinase transcriptionally-regulated early response gene, Homer1a, was up-regulated transiently in the hippocampus and down-regulated in the cortex of young adult male Fischer 344 X Brown Norway rats at 48 h after 40 Gy of fractionated whole-brain irradiation. Two months after fractionated whole-brain irradiation, these changes in Homer1a expression correlated with a down-regulation of the hippocampal glutamate receptor 1 and protein kinase C_γ, and an up-regulation of cortical glutamate receptor 1 and protein kinase C_γ. Two drugs that prevent radiation-induced cognitive impairment in rats, the angiotensin type-1 receptor blocker, L-158,809, and the angiotensin converting enzyme inhibitor, ramipril, reversed the fractionated whole-brain irradiation-induced Homer1a expression at 48 h in the hippocampus and cortex and restored glutamate receptor 1 and protein kinase C_γ to the levels in shamirradiated controls at 2 months after fractionated whole-brain irradiation. These data indicate that Homer1a is, (1) a brain region specific regulator of radiation-induced brain injury, including cognitive impairment and (2) potentially a druggable target for preventing it.

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INTRODUCTION

Over 250,000 patients per year receive fractionated partial irradiation or whole-brain irradiation (fWBI) for the treatment of primary or metastatic brain cancer (1, 2). The effectiveness of this treatment modality is limited by the radiation dose that can be safely delivered to the normal tissue adjacent to the tumor (3). The majority of patients that receive fWBI are at risk for developing late radiation-induced brain injury, which primarily consists of a progressive, irreversible cognitive impairment manifesting as a decrease in short-term memory, attention, concentration and/or language proficiency (3, 4). Although the exact mechanism(s) behind radiation-induced brain injury are unknown, radiation has been reported to increase microglia activation (5, 6) and decrease neurogenesis (7, 8), suggesting that neuroinflammation and impaired neurogenesis play a role.

Currently, there are no long-term treatments for the prevention of radiation-induced brain injury. However, preclinical studies have led to the development of several ongoing clinical trials. In rodent models of radiation-induced brain injury, the peroxisome proliferator activating receptor alpha (PPARα) agonist, fenofibrate and the anti-inflammatory drug, indomethacin, prevent radiation-induced decreases in hippocampal neurogenesis (9, 10), and fenofibrate prevents radiation-induced cognitive impairment (Dana Greene-Schloesser, personal communication). Additionally, partial restoration of neuronal populations by implantation of neural stem cells or embryonic stem cells has been reported to prevent radiation-induced cognitive impairment in nude rats (11, 12). As a result, radiotherapists are currently attempting to prevent radiation-induced brain injury by shielding the hippocampus (13), one of two sites of neurogenesis in the brain (14). However, hippocampal shielding has not always proven to be effective at preventing cognitive impairment, suggesting that other brain regions contribute to the development of radiation-induced brain injury (4).

Our laboratory has focused on the role of neuroinflammation in radiation-induced brain injury. *In vitro* studies have identified that radiation generates reactive oxygen species (15) and activates the MAP kinase mediated inflammatory response in brain cells (16, 17). Blocking radiation-induced MAP kinase signaling with either PPARα or PPARδ agonists (16, 18) or the renin-angiotensin system (RAS) heptapeptide, angiotensin-(1-7) (Elizabeth D. Moore, personal communication), inhibits the induction of inflammatory cytokines (e.g., Il-6, Cox-2, MCP-1) in cultured microglia or astrocytes. Furthermore, blockade of the RAS with the angiotensin type-1 receptor blocker (ARB), L-158,809 (19), or the angiotensin converting enzyme inhibitor (ACEI), ramipril (20), prevents fWBI-induced cognitive impairment, but does not protect fWBI-induced decreases in hippocampal neurogenesis in young adult male rats. Thus, the mechanism(s) for developing fWBI-induced brain injury, including cognitive impairment, and how to prevent it have not been fully elucidated.

Brain region specific radiation responses may partially account for the difficulty in elucidating the mechanism(s) for the development of fWBI-induced brain injury and generating a successful strategy to prevent it. For example, recent studies by Peiffer *et al*. have shown that fWBI-induced damage to brain regions other than the hippocampus may be able to predict which irradiated brain tumor patients will develop cognitive impairment (4). It has also been reported that radiation induces brain region specific changes in white matter

(21), acetylcholinesterase (22) and cerebral metabolism (23). Moreover, brain regions vary in their cell density, number and type (24). Consequently, these biochemical, cellular and structural variations may be responsible for the brain region specific radiation response that is observed in the clinic (4).

One of the most successful clinical trials for modulating radiation-induced cognitive impairment employed the acetylcholinesterase inhibitor, donepezil a drug predominantly used to treat mild to moderate Alzheimer's dementia (25, 26). Administration of donepezil for 24 weeks beginning 6 months after fWBI improved moderate dementia in 2/3 of the irradiated brain tumor patients (25). Radiation-induced brain injury and Alzheimer's disease (AD) also share pathological similarities, including changes in myelination, NMDA receptors and glutamate/glutamine levels (22, 26-28). This suggests that the molecular mechanism(s) responsible for the development of cognitive deficits in AD may also be involved in the development of radiation-induced cognitive impairment.

Activation of the ERK signaling pathway has been shown to result in the transcription of inflammatory cytokines in both AD (29) and a preclinical model of radiation-induced brain injury (30). Also, activation of ERK has been shown to result in the transcription of early response genes involved in synaptic plasticity, including a protein, Homer1a, that is only found in the nervous system (31). Homer1a mRNA levels have been shown to be elevated in AD and may contribute to the development of AD cognitive deficits (32). Homer1a is a truncated form of the long Homer1 protein, which contains both an EVH1 and carboxy-terminal domain. The Homer1 EVH1 domain binds to metabotropic glutamate family 1 receptors while the carboxy-terminal domain binds other proteins within the postsynaptic complex at the cell membrane (33). Homer1a lacks the carboxy-terminal domain. Thus, Homer1a binds solely to family 1 metabotropic glutamate receptors (mGluR) and inhibits their binding to the synapse. (34). Overexpression of Homer1a in the hippocampus is known to abolish maintenance of CA3-CA1 long-term potentiation (LTP) (35), synaptic plasticity (36, 37) and impair working memory (35). Radiation also alters LTP and impairs working memory (38). However, whether radiation modifies Homer1a expression in the brain has not yet been explored. In the current study, we tested the hypotheses that; (1) radiation alters Homer1a expression similar to what is observed in AD; and (2) Homer1a is potentially a druggable target for preventing radiation-induced brain injury, including cognitive impairment.

MATERIALS AND METHODS

Materials

The ARB, L-158,809, (Merck Pharmaceuticals, Whitehouse Station, NJ) was dissolved in water at a concentration of 20 mg/L and administered to rats in their drinking water. The ACEI, ramipril (Pfizer Inc., New York, NY) was also dissolved in water at a concentration of 15 mg/L and administered to rats in their drinking water. The following antibody concentrations were used in this study: 1:1,000 rabbit anti-mGluR1 (Millipore, Billerica, MA), 1:5,000 rabbit anti-p-GluR1 (Ser831; PhosphoSolutions, Aurora, CO), 1:4,000 rabbit-anti-p-GluR2 (Ser880; PhosphoSolutions), 1:2,000 rabbit anti-GluR2/3 (Millipore), 1:1,000 rabbit anti-PKCγ (Abcam, Cambridge, MA), 1:1,000 mouse-anti-p-ERK1/2 (Santa Cruz

Biotechnologies, Dallas, TX), 1:1,000 ERK1/2 (Cell Signaling, Beverly, MA) and 1:10,000 mouse anti-β-actin (Sigma-Aldrich, St. Louis, MO).

Animals

Forty-eight Fischer 344 x Brown Norway young adult (10–12-week-old) male rats were obtained from Harlan Sprague Dawley Inc. (Indianapolis, IN) and housed in pairs on a 12:12 light/dark schedule with access to food and water *ad libitum*. All animal handling and experiments were performed in strict accordance with the Declaration of Helsinki and the NIH Guide for Care and Use of Laboratory Animals as approved by the Wake Forest University School of Medicine Institutional Animal Care and Use Committee.

Experimental Design

After an acclimation period of 2 weeks, rats were randomized to 6 treatment groups (n = 8); Group 1: sham irradiation, Group 2: L-158,809 alone, Group 3: ramipril alone, Group 4: fWBI alone, Group 5: fWBI + L-158,809 and Group 6: fWBI + ramipril. Rats in Groups 2, 3, 5 and 6 were administered the drug 3 days before initiating fWBI, and then continuously for the duration of the experiment. Fresh drinking water with/without drug was supplied every other day.

Irradiation Procedure

A total dose of 40 Gy was delivered in 8 fractions of 5 Gy/fraction, twice per week for 4 weeks as described previously (39). Briefly, all irradiations were performed in a 267 TBq (7,214 Ci) self-shielded ^{137}Cs irradiator using lead and Cerrobend shielding devices to collimate the beam so that the whole brain, including the brain stem, was irradiated. The average dose rate to the midline of the brain was ~4 Gy/min; the eyes and body received ~15% and ~3% of the brain dose, respectively. To ensure that the rats received the same midline brain dose, each lightly anesthetized [ketamine (75 mg/kg)/xylazine (7 mg/kg)] rat had the twice-weekly dose delivered to alternate sides of the head on alternate days. Sham-irradiated rats were anesthetized at the same time as the fWBI rats.

Tissue Collection

Four rats from each treatment group were euthanized 48 h (24 rats) or 2 months (24 rats) after the completion of fWBI. The brains were removed rapidly and grossly dissected into cortex and hippocampus. The sections were then flash frozen in liquid nitrogen and stored at −80°C.

Immunoblot Hybridization

Total cellular protein was harvested from the frozen hippocampal and cortical tissues using M-PER lysis buffer (Pierce Biotechnology, Rockford, IL) supplemented with 1 mg/mL aprotinin (Sigma-Aldrich), 1 m*M* leupeptin (Sigma-Aldrich), 10 mg/mL phenylmethylsulfonyl fluoride (PMSF), 1 m*M* Na_3VO_4 (Sigma-Aldrich), and 150 m*M* NaCl. After homogenization, the tissue lysates were centrifuged at 12,500 rpm for 30 min and the supernatant collected. Protein concentrations were measured using the Bradford assay (Bio-Rad, Hercules, CA) at an absorbance 595 nm. Aliquots (25–30 μg) of protein

were loaded onto a 10% polyacrylamide gel and the protein separated by SDS-PAGE electrophoresis. The separated proteins were transferred to polyvinylidene difluoride membranes (Life Technologies) at 35 V overnight, blocked in 5% milk in TBST (0.02 *M* Tris, 0.015 *M* NaCl, 0.05% Tween 20, pH 7.5) and incubated overnight with the desired primary antibody. The membranes were then washed, incubated with the appropriate horseradish peroxidase-conjugated secondary antibody and developed using the ECL reagent (GE Healthcare, Piscataway, NJ) and a Kodak film processor (Rochester, NY). Films were scanned and densitometry performed to quantify the protein using Adobe Photoshop Elements 6.0. All protein levels were expressed as fold changes, with beta actin used as the loading control.

RNA isolation and Quantitative Real Time Polymerase Chain Reaction with TaqMan

RNA was harvested from the frozen hippocampal and cortical tissue using Trizol reagent (Invitrogen, Grand Island, NY) according to the manufacturer's protocol. DNA contamination was removed by chloroform extraction (Ambion, Austin, TX). Real-time PCR amplifications were conducted in a 20 μl reaction volume containing 2 μl cDNA, 10 μl TaqMan Master Mix (Life Technologies Applied Biosystems), 0.1 μ*M* Homer1a specific probe, upstream primer and downstream primer (Life Technologies Applied Biosystems) and 7 μl nuclease-free water. Real-time PCR was carried out in an ABI Prism 7,000 at 50°C for 2 min, 95°C for 2 min, and 45 cycles of 95°C for 15 min, 55°C for 30 s and 72°C for 30 s. The fold changes in Homer1a gene expression were calculated using the comparative Ct method (40).

Statistical Analysis

All data are expressed as the mean and ±SEM. All analyses were carried out using GraphPad 5.0 (GraphPad Prism Software). Analyses of the experiments comparing irradiated data to sham-irradiated data were performed using a Student's *t* test for equal sample sizes. Analyses to determine if there was a radiation or drug effect were performed using 2-way ANOVAs; group comparisons were measured using Bonferroni post-hoc tests. All results were considered statistically significant at the $P < 0.05$ level.

RESULTS

fWBI Alters Homer1a Expression in the Hippocampus and Cortex of Young Adult Male Rats

To determine if fWBI alters Homer1a expression in the brain similar to what is observed in AD, we measured the Homer1a expression in both the hippocampus and cortex at 48 h and 2 months after fWBI. Homer1a expression increased ~2-fold in the hippocampus and decreased ~50% in the cortex at 48 h after fWBI compared to sham-irradiated controls (Fig. 1A). At 2 months after fWBI Homer1a expression was decreased ~70% in the hippocampus and ~50% in the cortex compared to sham-irradiated controls (Fig. 1B). Homer1a is known to be regulated by ERK activation (41) and single doses of irradiation have been shown to activate ERK *in vitro* (16, 17) and *in vivo* (30). Consequently, we measured p-ERK in the hippocampus and cortex to determine if fWBI modulation of Homer1a may be associated with the activation of ERK at 48 h and 2 months. fWBI-induced an ~2-fold increase in p-

ERK in the hippocampus and a ~50% decrease in p-ERK in the cortex at both 48 h (Fig. 1C) and 2 months (Fig. 1D) after fWBI compared to sham-irradiated controls. Taken together, these data suggest that fWBI alters Homer1a expression in a brain region specific manner, consistent with the brain region specific radiation responses observed in preclinical (22, 23) and clinical studies (4, 21).

mGluR1 and its Downstream Effector, PKCγ, are Decreased in the Hippocampus at 2 Months after fWBI

Homer1a binds to mGluR1 and inhibits mGluR1 binding to the synapse (42). Homer1a overexpression is associated with decreased LTP and working memory (35). Finally, hippocampal decreases in mGluR1 and its downstream effectors have been reported to be associated with a decline in cognition (43). Consequently, we measured the total levels of hippocampal mGluR1 and its downstream effector, PKCγ, a kinase that has been shown to be activated by Ca^{2+} release from mGluR1 complexes at 2 months after fWBI (42). Total hippocampal mGluR1 levels decreased ~80% at 2 months after fWBI compared to sham-irradiated controls (Fig. 2A). Furthermore, changes in mGluR1 activation have been shown to alter phosphorylation of AMPA GluR1 at Ser831 (44). However, at 2 months after fWBI, we did not detect a change in the levels of p-GluR1 (Fig. 2B) or changes in other AMPA receptors, total AMPA GluR2/3 (Fig. 2C) and AMPA p-GluR2 (Fig. 2D), suggesting that fWBI only alters the total levels of mGluR1. Also, fWBI induced an ~60% decrease in the total levels of the downstream effector, PKCγ (Fig. 2E), suggesting that a decrease in mGluR1 levels has the potential to produce a decrease in mGluR1 downstream signaling after fWBI. These data suggest that fWBI produces an early increase in hippocampal Homer1a expression (Fig. 1A and B), which correlates with a decrease in hippocampal mGluR1 (Fig. 2A) and its downstream effectors at 2 months after fWBI (Fig. 2E), similar to what is found in other neurodegenerative conditions such as epileptic seizures (45, 46), Huntington's disease (47) and AD (32, 48).

mGluR1 and its Downstream Effector, PKCγ, are Increased in the Cortex at 2 Months after fWBI

Unlike the hippocampus, the total levels of cortical mGluR1 increased ~2-fold (Fig. 3A) and its downstream effector, PKCγ, increased ~3-fold (Fig. 3E) compared to sham-irradiated controls at 2 months after fWBI. Similar to the hippocampus, fWBI did not alter the total levels of cortical p-GluR1 (Fig. 3B), GluR2/3 (Fig. 3C) or p-GluR2 (Fig. 3D). These data suggest that fWBI produces a sustained decrease in cortical Homer1a expression (Fig. 1A, B) which correlates with an increase in cortical mGluR1 (Fig. 3A) and its downstream effectors at 2 months after fWBI (Fig. 3E), similar to that reported in mouse models of aging (49).

L-158,809 and Ramipril Prevent the Changes in Hippocampal Homer1a Expression and its Downstream Signaling after fWBI

Blocking the RAS with the ARB, L-158,809 or the ACEI, ramipril, has been shown to prevent fWBI-induced hippocampal-independent cognitive impairment (19, 20). In the present study, both L-158,809 and ramipril prevented the fWBI-induced increase in hippocampal Homer1a expression at 48 h after fWBI (Fig. 4A), but only L-158,809 had the

same effect at 2 months (Supplementary Fig. S1: http://dx.doi.org/10.1667/RR13475.1.S1). These RAS blockers restored the hippocampal mGluR1 levels to the sham-irradiated control levels at 2 months (Fig. 4B). Similarly, both drugs prevented the fWBI-induced changes in p-ERK (Fig. 4C) upstream of Homer1a and PKCγ (Fig. 4D) downstream of mGluR1. L-158,809 and ramipril did not affect the levels of p-GluR1 (Supplementary Fig. S2A; http://dx.doi.org/10.1667/RR13475.1.S1), total GluR2/3 (Supplementary Fig. S2B; http://dx.doi.org/10.1667/RR13475.1.S1) or p-GluR2 (Supplementary Fig. S2C; http://dx.doi.org/10.1667/RR13475.1.S1). These data suggest that L-158,809 and ramipril are able to prevent the early fWBI-induced increase in hippocampal Homer1a expression and restore the mGluR1 signaling, which is likely important for hippocampal-dependent cognitive function.

L-158,809 and Ramipril Prevent the Changes in Cortical Homer1a Expression and its Downstream Signaling after fWBI

Similar to the hippocampus results, both L-158,809 and ramipril prevented the decrease in cortical Homer1a expression at 48 h after fWBI (Fig. 5A), but only L-158,809 had the same effect at 2 months (Supplementary Fig. S3; http://dx.doi.org/10.1667/RR13475.1.S1). These RAS blockers restored the cortical mGluR1 levels to the sham-irradiated control levels at 2 months after fWBI (Fig. 5B). Similarly, both drugs prevented the fWBI-induced changes in the upstream and downstream proteins, p-ERK (Fig. 5C) and PKCγ (Fig. 5D), respectively. L-158,809 and ramipril did not affect the levels of p-GluR1 (Supplementary Fig. S4A; http://dx.doi.org/10.1667/RR13475.1.S1), total GluR2/3 (Supplementary Fig. S4B; http://dx.doi.org/10.1667/RR13475.1.S1) or p-GluR2 (Supplementary Fig. S4C; http://dx.doi.org/10.1667/RR13475.1.S1). These data suggest that L-158,809 and ramipril are able to prevent the early fWBI-induced decrease in cortical Homer1a expression and restore mGluR1 signaling which is likely important for cortical-dependent cognitive function.

DISCUSSION

The majority of brain tumor patients surviving >6 months after radiation therapy will develop late radiation-induced brain injury predominantly manifesting as a progressive, irreversible cognitive impairment. In the study reported here, we demonstrated that the ERK transcriptionally-regulated early response gene, Homer1a, is differentially regulated in the hippocampus and cortex of young adult male rats at 48 h after a total 40 Gy dose of fWBI (Fig. 1A). Furthermore, treatment with L-158,809 or ramipril, drugs known to prevent fWBI-induced cognitive impairment in rats (19, 20), prevented fWBI-induced changes in Homer1a expression in both the hippocampus and the cortex at 48 h after fWBI (Figs. 4A and 5A), but only L-158,809 had the same effect at 2 months (Supplementary Figs. S1 and S3; http://dx.doi.org/10.1667/RR13475.1.S1). Homer1a belongs to a family of scaffolding proteins that localize in the synapse and regulate intracellular calcium homeostasis (50, 51), gene transcription, signal transduction and receptor trafficking (34, 41). The longer forms of Homer1 are constitutively expressed and have two functional domains, (1) the EVH1 domain which binds to the Shank, mGluR1/5 and ryanodine receptors; and (2) a coiled coil structure which binds to other Homer forms (34). The Homer1a form lacks the coiled coil domain and disrupts both the scaffolding and signaling capabilities of the long forms of

Homer proteins by competitively binding to the Shank-mGluR1/5-ryanodine complex (41, 42).

Homer1a is transcriptionally induced in neurons after seizures (45, 52) and in the hippocampus during LTP (53–55). Homer1a regulates activity-induced post and pre-synaptic remodeling (56) and dendritic axonal targeting of mGluR5 (57). Overexpression of Homer1a in the rodent hippocampus or striatum impairs hippocampal-dependent memory and motor performance in behavioral tasks (31). Homer1a expression is increased in AD (32) and animal models of epileptic seizures (58) and Huntington's disease (41). Homer1 knockout mice exhibit a schizophrenic-like phenotype with behavioral (motivational and emotional), cognitive, sensorimotor processing and glutamatergic abnormalities (59, 60). In a rat model of aging, increased expression of Homer1a and decreased mGluR5 signaling in the hippocampus were associated with cognitive deficits (61). Conversely, decreased Homer1a expression in the whole brain (largely cortical expression) was also associated with a loss of cognitive and motor function in mice (49). These data demonstrate that, depending on the brain region involved, both increased and decreased Homer1a expression can lead to cognitive impairment. Our data showing that Homer1a is differentially expressed in the hippocampus and cortex after fWBI (Fig. 1A) is consistent with this concept.

The reason for the differential regulation of Homer1a expression in the hippocampus and cortex is not clear. We speculate that the brain microenvironment is likely to contribute to this differential signaling. Brain regions vary in their cellular distribution (24), and this may contribute to their response to different stimuli (62, 63). Moreover, it has been shown that astrocytes, microglia and neurons isolated from different brain regions respond differently to injury (64-66). Therefore, it is not unique that fWBI induces specific brain region-mediated responses in Homer1a expression.

The study reported here indicates that fWBI induces changes in p-ERK, Homer1a, mGluR1 and PKCγ (Figs. 1-3). In the hippocampus, fWBI increased phosphorylation of ERK and transcription of Homer1a at 48 h after fWBI (Fig. 1A, C). This increase in early hippocampal Homer1a expression correlated with a decrease in mGluR1 (Fig. 2A) and its downstream effector, PKCγ (Fig. 2E) at 2 months after fWBI. In contrast to the hippocampus, fWBI decreased p-ERK and Homer1a expression at 48 h in the cortex (Fig. 1A, D). This was concomitant with a large increase in mGluR1 (Fig. 3A) and PKCγ (Fig. 3B) at 2 months after fWBI. fWBI did not alter p-GluR1, total GluR2/3 or p-GluR2 at 2 months after fWBI (Supplementary Figs. S2 and S4; http://dx.doi.org/10.1667/RR13475.1.S1). Although Homer1a has also been shown to regulate phosphorylation of GluR1 (44) and GluR2 (50), our data suggests that Homer1a is regulating mGluR1 signaling after fWBI. At 2 months after fWBI, only L-158,809 prevented the fWBI-induced changes in Homer1a expression in both the hippocampus and cortex (Supplementary Figs. S1 and S3; http://dx.doi.org/10.1667/RR13475.1.S1). In addition, ERK activation does not match Homer1a regulation in the hippocampus at 2 months after fWBI, likely due to ERK's involvement in many other processes (55, 67, 68). Therefore, we suspect that the role of Homer1a in fWBI-induced cognitive impairment appears to be limited to an "early" event after irradiation.

Memory/learning paradigms suggest that trafficking of mGluR1 from an intracellular compartment to the plasma membrane is an early event during neural plasticity (43). Thus, the changes in mGluR1 (Figs. 2A, 3A) at 2 months after fWBI may result in alterations in hippocampal and/or cortical neural plasticity. Of note, our rat model of fWBI-induced brain injury does not exhibit hippocampal-independent cognitive impairment until 6 months after fWBI, similar to what is observed clinically (3). Given that these hippocampal and cortical fWBI-induced early changes in Homer1a expression and delayed changes in mGluR1 signaling were prevented by L-158,809 and ramipril, drugs that also prevent fWBI-induced hippocampal-independent cognitive impairment (19, 20), we speculate that this signaling mechanism is important for radiation-induced cognitive impairment.

It is important to note that all of the data in this study describing the relationship between Homer1a expression and mGluR1 signaling is correlative rather than causative. Unfortunately, our analysis was limited to Homer1a expression because a suitable antibody is not available for measuring Homer1a protein. Future experiments that measure Homer1a expression in the hippocampus and cortex over time periods up to 6 months after fWBI will be required to elucidate the role of Homer1a and mGluR1 signaling in fWBI-induced cognitive impairment. Finally, fWBI studies with Homer1a knockout mice (50) could further our understanding of the role of Homer1a and mGluR1 signaling in fWBI-induced cognitive impairment.

The ARB, L-158,809, and the ACEI, ramipril, both prevent fWBI-induced cognitive impairment in rats (19, 20) and inhibit the early fWBI-induced differential expression of Homer1a in the hippocampus (Fig. 4A) and cortex (Fig. 5A). Although ramipril ameliorates radiation-induced neuroinflammation, our results suggest that ramipril may have other targets for preventing fWBI-induced cognitive impairment (20). The data reported here indicate that components of the p-ERK-Homer1a-mGluR1-PKCγ signaling pathway may be druggable candidates for preventing fWBI-induced cognitive impairment. ERK is the first member of the pathway and has been shown to be activated both *in vitro* (16, 17) and *in vivo* (30) by ionizing radiation. Moreover, ERK inhibition has been associated with decreases in radiation-induced neuroinflammation (16, 17,30). In the study reported here, both L-158,809 and ramipril inhibited fWBI-induced changes in p-ERK. However, ERK is not an attractive druggable target to prevent fWBI-induced brain injury because ERK is involved in numerous biochemical and cellular processes in several organs (68). Thus, inhibition of ERK is likely to have negative effects on one or more organ systems in the body (69). To our knowledge, there are no reports of Homer1a having an important function outside the nervous system (34). Consequently, this makes Homer1a an attractive druggable target for the prevention of fWBI-induced brain injury, including cognitive impairment.

In summary, Homer1a, a protein only expressed in the nervous system, was up-regulated in the hippocampus and down-regulated in the cortex of young adult male rats after fWBI, similar to what is observed in other neurodegenerative conditions. These early changes in Homer1a expression resulted in altered mGluR1 and PKCγ levels in both the hippocampus and cortex at 2 months after fWBI. Furthermore, treatment with L-158,809 or ramipril, which prevent fWBI-induced cognitive impairment in rats, restored early Homer1a expression as well as mGluR1 and its downstream effector levels to those in sham-irradiated

controls. Taken together, these data suggest that early Homer1a expression, (1) plays an important role in the development of fWBI-induced brain injury, including cognitive impairment, and (2) is potentially a druggable target for preventing it.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the NIH grant CA112593 (MER) and CA122318 (MER) from NCI.

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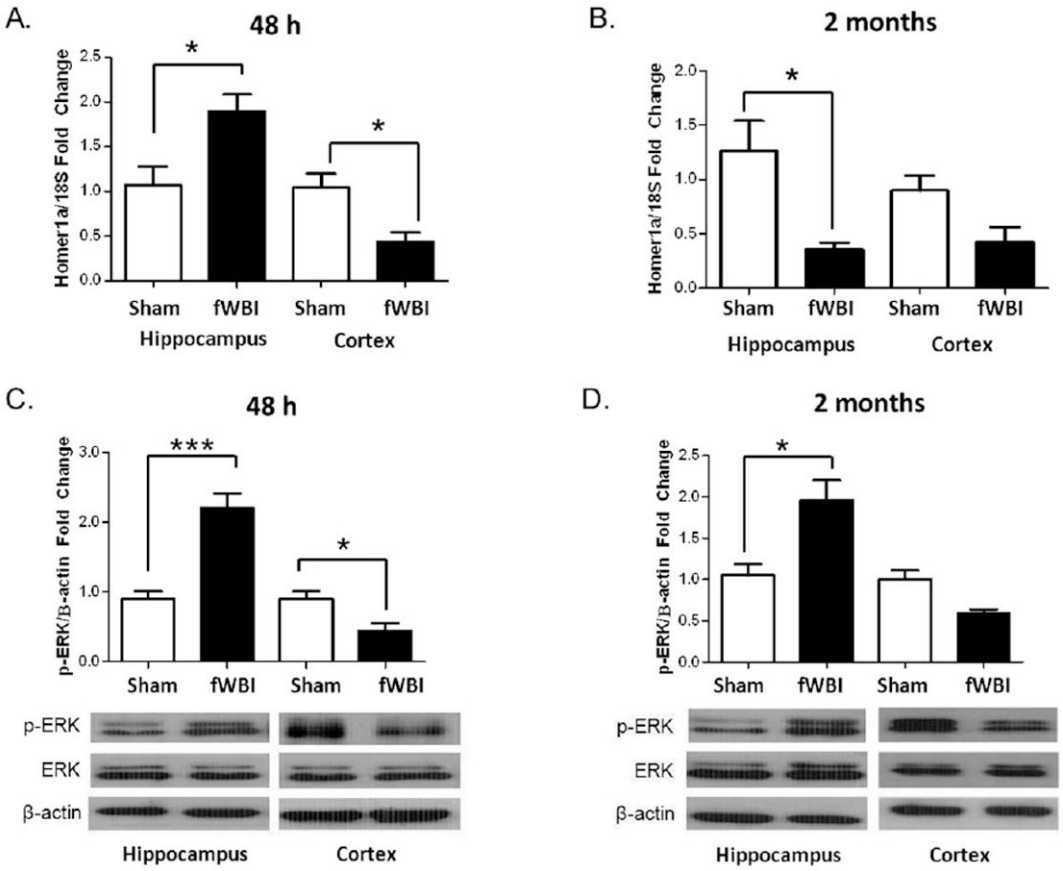

FIG. 1.

fWBI alters Homer1a expression in the hippocampus and cortex of young adult male rats. Hippocampal and cortical Homer1a mRNA levels were determined by TaqMan real time PCR on mRNAs isolated 48 h (panel A) or 2 months (panel B) after fWBI and the data normalized to 18S mRNA levels. Hippocampal and cortical p-ERK proteins were analyzed by Western blot hybridization of lysates isolated 48 h (panel C) and 2 months (panel D) after fWBI; β-actin was the loading control. Data are expressed as the mean ± SEM from 4 rats. (*$P < 0.05$, ***$P < 0.001$)

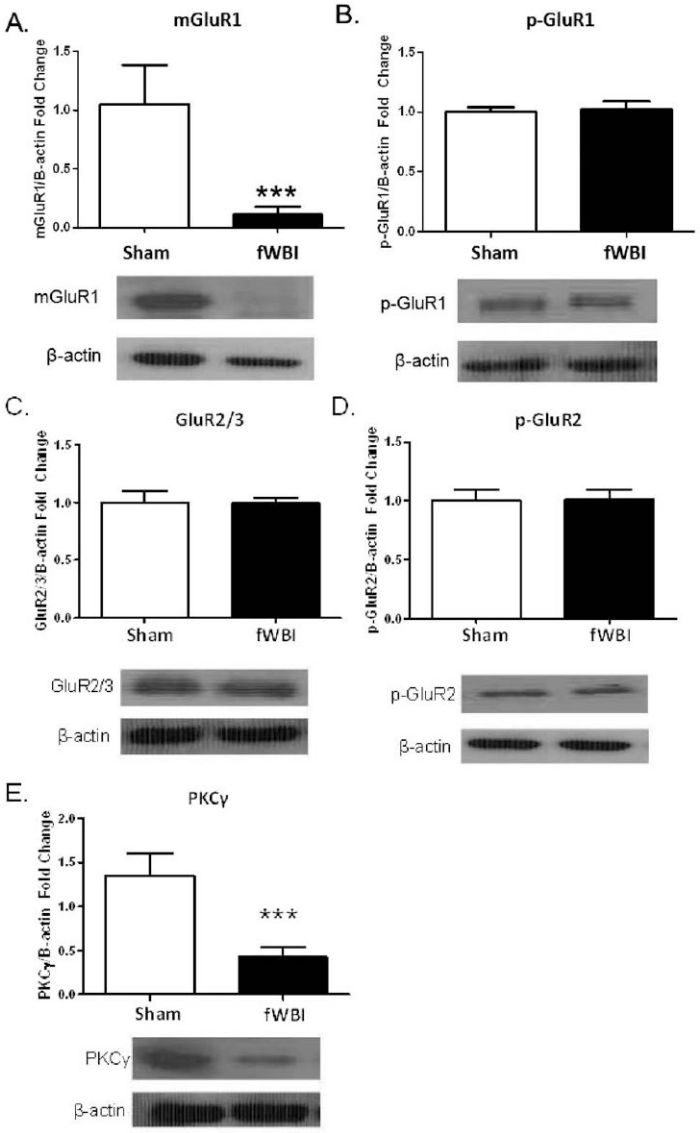

FIG. 2.

mGluR1 and its downstream effector, PKCγ, are decreased in the hippocampus at 2 months after fWBI. Total mGluR1 (panel A), p-GluR1 (panel B), total GluR2/3 (panel C), p-GluR2 (panel D) and total PKCγ (panel E) protein levels were analyzed by Western blot hybridization at 2 months after fWBI; β-actin was the loading control. Data are expressed as the mean ± SEM from 4 rats. (***$P < 0.001$)

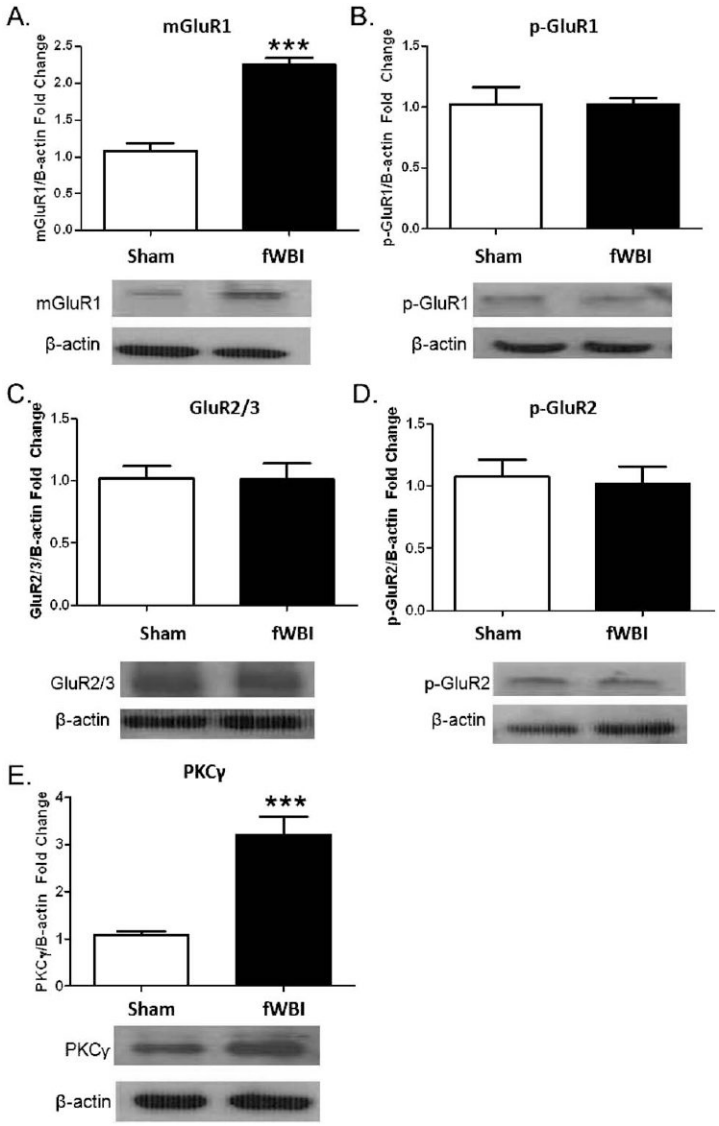

FIG. 3.

mGluR1 and its downstream effector, PKCγ, are increased in the cortex at 2 months after fWBI. Total mGluR1 (panel A), p-GluR1 (panel B), total GluR2/3 (panel C), p-GluR2 (panel D) and total PKCγ (panel E) protein levels were analyzed by Western blot hybridization at 2 months after fWBI; β-actin was the loading control. Data are expressed as the mean ± SEM from 4 rats. (***$P < 0.001$)

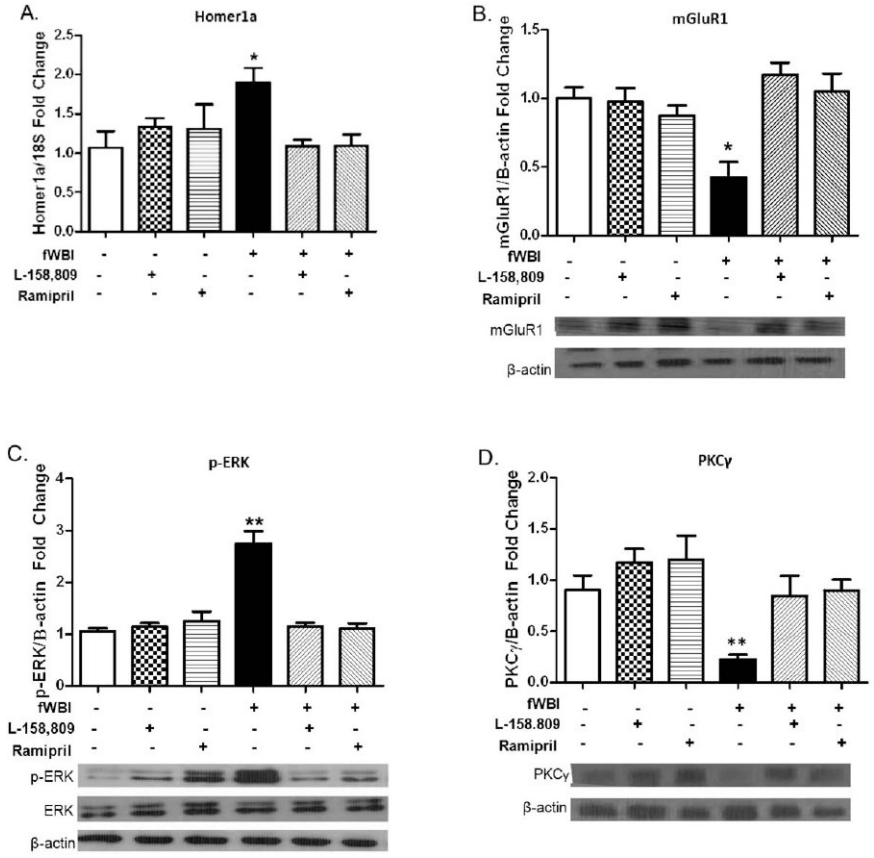

FIG. 4.

L-158,809 and ramipril prevent the changes in hippocampal Homer1a expression and its downstream signaling after fWBI. Homer1a mRNA levels were determined by TaqMan real time PCR on mRNAs isolated 48 h after fWBI, and the data normalized to 18S mRNA levels (panel A). Total mGluR1 at 2 months after fWBI (panel B), p-ERK at 48 h after fWBI (panel C) and total PKCγ at 2 months after fWBI (panel D) protein levels were analyzed by Western blot hybridization; β-actin was the loading control. Data are expressed as the mean ± SEM from 4 rats. (**P* < 0.05, ***P* < 0.01)

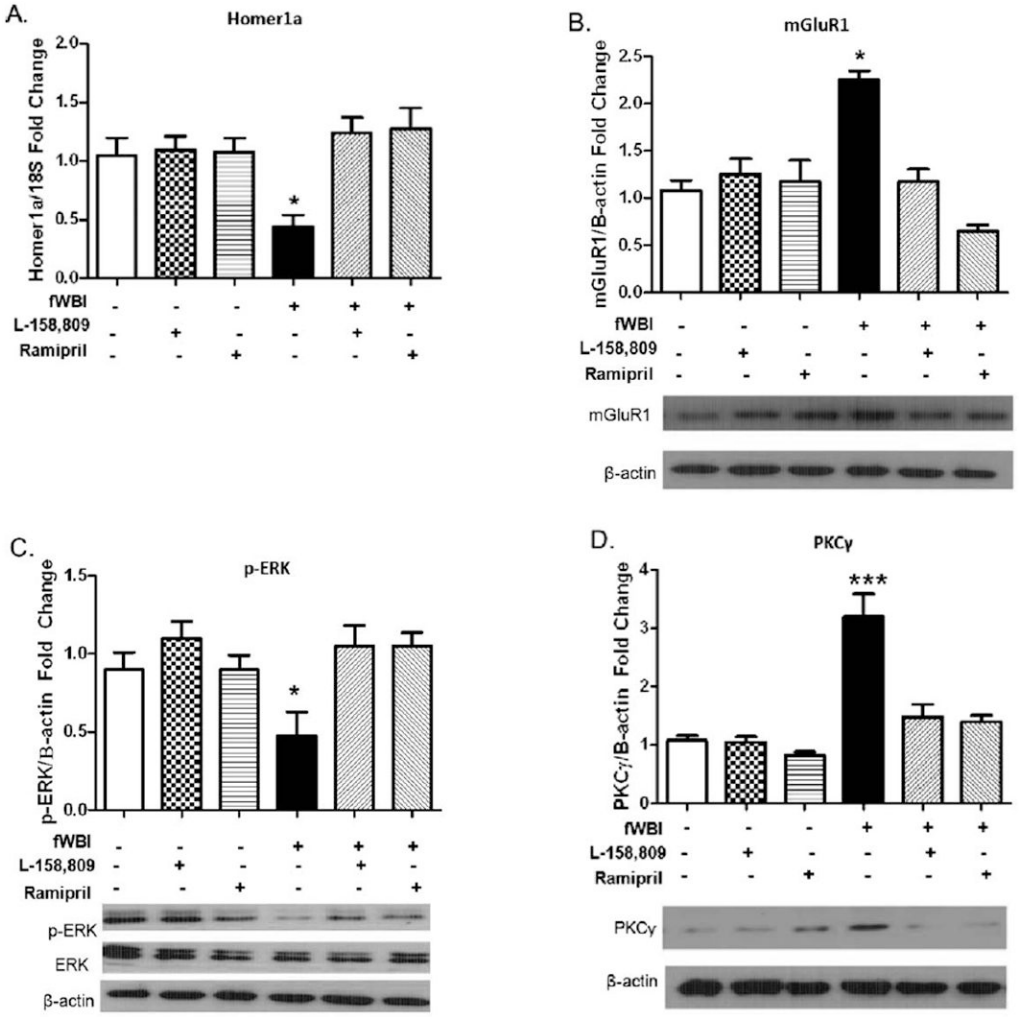

FIG. 5.

L-158,809 and ramipril prevent the changes in cortical Homer1a expression and its downstream signaling after fWBI. Homer1a mRNA levels were determined by TaqMan real time PCR on mRNAs isolated 48 h after fWBI, and the data normalized to 18S mRNA levels (panel A). Total mGluR1 at 2 months after fWBI (panel B), p-ERK at 48 h after fWBI (panel C) and total PKCγ at 2 months after fWBI (panel D) protein levels were analyzed by Western blot hybridization; β-actin was the loading control. Data are expressed as the mean ± SEM from 4 rats. (*$P < 0.05$, ***$P < 0.001$)