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Reversal of Trauma-Induced Amnesia in Mice by a Thrombin Receptor Antagonist

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Abstract Minimal traumatic brain injury (mTBI) is associated with the existence of retrograde amnesia and microscopic bleeds containing activated coagulation factors. In an mTBI model, we report that thrombin induces amnesia through its receptor protease-activated receptor 1 (PAR-1). Thrombin activity was significantly elevated (32 %, $p<0.05$) 5 min following mTBI compared to controls. Amnesia was assessed by the novel object recognition test in mTBI animals and in animals injected intracerebroventricularly (ICV) with either thrombin or a PAR-1 agonist 1 h after the acquisition phase. Saline-injected controls had a preference index of over 0.3 while mTBI animals and those injected with thrombin or the PAR-1 agonist spent equal time with both objects indicating no recall of the object presented to them 24 h previously ($p<0.05$). Co-injecting a PAR-1 antagonist (SCH79797) completely blocked the amnestic effects of mTBI, thrombin, and the PAR-1 agonist. Long-term potentiation, measured in hippocampal slices 24 h after mTBI, ICV thrombin or the PAR-1 agonist, was significantly impaired and this effect was completely reversed by the PAR-1 antagonist. The results support a crucial role for PAR-1 in the generation of amnesia following mTBI, revealing a novel therapeutic target for the cognitive effects of brain trauma.

Keywords mTBI · Amnesia · Thrombin · PAR1 · Hippocampus · Long term potentiation

Introduction

Minimal traumatic brain injury (mTBI) in humans can be identified as a pathological condition when it concurs with the clinical manifestation of concussion and is sometimes associated with the post-concussion syndrome (PCS). Known since ancient times (Feinsod and Langer 2012) mild traumatic brain injury and concussion are defined by transient loss of consciousness, amnesia, altered mental status following an acute closed head injury. Most patients recover quickly, with a predictable clinical course of recovery within the first 1 to 2 weeks following traumatic brain injury (Mott et al. 2012). A prominent manifestation of PCS is long-term memory loss and at the initial phase of the trauma, there is commonly both retrograde and anterograde amnesia (Shaw 2002).

Animal model studies of mTBI have shown micro-vascular damage and other microscopic findings associated with brain tissue damage (Povlishock and Christman 1995; Povlishock and Jenkins 1995). It has been suggested that following the vascular injury, there is extravasation of blood components, such as coagulation factors, which might protect against uncontrolled extensive brain hemorrhage. Alternatively, others have attributed many of the long-term effects of brain trauma to the effects of thrombin, the central protease in blood coagulation (Mhatre et al. 2004).

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Elucidation of the biological basis of mTBI requires an animal model which would be able to reproduce the behavioral and cognitive deficits observed in humans. Such a model has been successfully developed and is based on closed head mTBI induced by free weight drop (Zohar et al. 2003, 2006; Aronovich et al. 2005; Milman et al. 2005; Tweedie et al. 2007). The most prominent biological findings in this model are diffuse axonal injury and various apoptotic changes (Tweedie et al. 2007; Tashlykov et al. 2007; Ucar et al. 2006; Rubovitch et al. 2011). In addition, morphological change in vascular permeability of the brain tissue has been observed in human PCS patients including alterations in cortical perfusion and blood–brain barrier (BBB) disruption (Korn et al. 2005). Similar changes in BBB permeability have been observed in our mouse model of mTBI (Pan et al. 2003), and such BBB disruption may well lead to serum components leakage into brain tissue. One of the key components of serum is the serine protease thrombin. Thrombin, beyond its pivotal role in the coagulation cascade, has been implicated in various processes in the central nervous system including neuroprotection, neurotoxicity (also in TBI models) and epilepsy (Donovan and Cunningham 1998; Friedmann et al. 1999, 2001a, b; Lee et al. 1997; Xue and Del Bigio 2001; Maggio et al. 2008, 2013a, b; Beilin et al. 2001, 2005, 2007; Movsesyan et al. 2001; Chapman 2006; Shavit et al. 2011). Biological activity of thrombin in cells is mediated by a family of unique protease-activated receptors (PARs) 1 through 4, with PAR-1 being the most abundant thrombin-activated receptor in the CNS (Luo et al. 2007). Thrombin activity is inhibited by various endogenous protease inhibitors, such as protease nexin-1 (PN-1), the amyloid precursor protein (APP) and antithrombin (AT), and elevations in their levels, were observed in the experimental animal model of CNS autoimmune disorders associated with BBB disruption (Beilin et al. 2001, 2005, 2007).

A major pathway by which thrombin affects neural tissue is through PAR-1 which is found on astrocyte endfeet localized at the synapse and on some neurons (Han et al. 2011; Shavit et al. 2011). A number of studies have found that thrombin acting through PAR-1 induces significant electrophysiological effects at the synapse including glutamate release, *N*-methyl-D-aspartate activation, depolarization, and long-term potentiation (LTP; Lee et al. 2007; Maggio et al. 2008, 2013c; Shavit et al. 2011). Although initially associated with excitation, PAR-1 activation results in a subsequent loss of the ability to generate further LTP (Reeves et al. 1995; Maggio et al. 2008, 2013c). The purpose of this study was to evaluate the hypothesis that amnesia generated immediately following mTBI is mediated by thrombin through PAR-1. In this study, we present evidence that thrombin activity increases briefly following mTBI and this increase is causally associated with the induction of amnesia through the thrombin receptor PAR-1.

Materials and Methods

Experimental Design

The experiment was divided into three arms:

(1) Biochemical evaluation of thrombin and its receptor (PAR-1) levels in brain slices and brain homogenates of the animals at different time points (5 min, 1 h) following mTBI induction;
(2) Behavioral assessment of cognitive performance of the animals following mTBI induction and/or pharmacological intervention by PAR-1 activation and blockade;
(3) Electrophysiological assessment of LTP formation ability in brain slices of the animals following mTBI induction and/or pharmacological intervention by PAR-1 activation and blockade;

Animals

Male ICR mice (8- to 10-week-old) weighing 30–40 g (Harlan, Jerusalem, Israel) were housed in standard conditions. Mice were fed a regular diet and given water without antibiotics. All possible measures were taken to avoid unnecessary suffering of the animals. The experiments were approved by the Tel Aviv University welfare review board.

mTBI Model in Mice

mTBI was induced using a free weight drop concussive device previously described (Zohar et al. 2003; Pan et al. 2003). Briefly, the device consisted of an 80-cm-high metal tube (13 mm in diameter) placed vertically over the head of the mouse. Minutes prior to the injury, the animals were slightly anesthetized by isoflurane (gaseous). Trauma was induced by a 30 g metal weight dropped down the metal tube on the right anterolateral side of the head (just anterior to the right ear). The mouse was placed on a sponge immobilization board which allowed head rotation following the impact thus mimicking the natural condition of head rotation in a whiplash injury. Following the injury the animals were sacrificed at different time points (5 min, 1 h) for further biochemical or electrophysiological evaluation and analysis or subjected to behavioral testing, some following intra-cerebro-ventricular injections.

Thrombin-Like Activity in Brain Slices Following mTBI

Prior to the harvest, the animals were anesthetized with pentobarbital and perfused transcardially with a PBS (pH7.4) solution. Following excision, control and mTBI brains were frozen in dry ice pre-chilled isopentane. Thereafter, coronal

slices were cut at 50 μm using a cryostat and then stored at -20 °C in a cryoprotectant buffer (24 % Glycerol, 28 % ethylene–glycol) until used for thrombin-like activity evaluation. Thrombin-like activity was assessed by the cleavage of a fluorogenic thrombin substrate (excitation 360 nm, emission 465 nm; Bachem I-1560, 0.2 mg/ml). The reactions were carried out in a black Nuncan 96-well microplate, using two slices/well. All the reactions were performed in TRIS buffer (150 mM NaCl, 1 mM $CaCl_2$, 50 mM TRIS–HCl: pH 8.0) containing 0.1 % BSA. All the reactions were performed in the presence of bestatin (0.1 mg/ml) to eliminate the effect of aminopeptidases and endopeptidases on the assay (Beilin et al. 2001; Shavit et al. 2011). Selected reactions were performed in the presence of 1 μM NAPAP (a highly specific thrombin inhibitor) to evaluate the specific contribution of thrombin to substrate cleavage generated in the slices. For calibration, known concentration of bovine thrombin (T-4648, Sigma) with the addition of 10 μl cryoprotectant buffer were used in the same assay.

Western Blot Analysis of Brain Homogenates

To assess PAR-1 and PN-1 levels in mouse brains, the animals were anesthetized with pentobarbital and perfused transcardially with a PBS (pH 7.4) solution. Brains were excised without the cerebellum, frozen in liquid nitrogen and kept in -80 C. Thereafter, the brains were homogenized in ten volumes of PBS supplemented with a protease inhibition (Sigma) using a Teflon pestle homogenizer. The homogenates were then centrifuged for 10 min at $400\times g$, the pellet was centrifuged again for 10 min at $900\times g$.The pellet was then incubated following pipetation for 5 min in ice cold lysis buffer [20 mM TRIS–HCl, pH 7.7, 10 mM KCl, 0.1 mM EDTA, 1.5 mM MgCl2, 0.2 % NP-40 and protease inhibition mixture (as described above)] and centrifuged again for 5 min at $3{,}500\times g$. Protein concentration in supernatant (cytosolic and membrane fractions) was determined using the BCA method. Proteins from the homogenates (12–20 μg total protein per lane) were separated by polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes for Western blot analysis (Towbin et al. 1979). Membranes were incubated overnight at 4 °C with primary rabbit anti PAR-1 antibody (diluted 1:500, Abcam ab32611) or primary goat anti PN-1 antibody (dilution range 1:200–1:300, Santa Cruz sc32454). Membranes were then incubated at room temperature for 1 h with horseradish peroxidase conjugated anti-rabbit or anti-goat antibody (Jackson Immunoresearch Laboratories) and bound antibody detected using enhanced chemoluminescense (ECL) assay kit (Pierce). ECL signal was detected by a computer based camera (Kodak, K-440), and the densitometry analysis of the detected signal was made using ImageJ software (NIH).

ICV Injections

To assess mouse brain in vivo responsiveness to PAR-1 activation or blockade, animals were anesthetized using an anesthesia system based on gaseous isofluorane. The skull was carefully exposed, and a small hole was formed with a 25-gauge needle above the right lateral ventricle (2 mm lateral to the midline and 2.5 mm posterior to the bregma). A 27-gauge needle attached to a Hamilton syringe was inserted at this point to a depth of 2 mm, where preliminary tests confirmed accurate intracerebroventricular (ICV) placement by injection of dye. One microliter of solution was then slowly infused, the needle was withdrawn, and the skin over the scalp was sutured. The injected solution contained either normal saline (NaCl 0.9 %), 2 unit/ml of bovine thrombin (T-4648, Sigma), 2 unit/ml thrombin mixed with 1 μM PAR-1 antagonist (SCH79797, referred to as SCH here, Tocris Bioscience), 100 μM PAR-1 agonist SFLLRN (Trap 14, Bachem, H-8105,) or 100 μM, PAR-1 agonist SFLLRN mixed with 1 μM PAR-1 antagonist or 1 μM PAR-1 antagonist alone. Following the injections, the animals were subjected to behavioral tests or sacrificed for electrophysiological evaluation.

Behavior

Neurological Assessment

The neurological status of each one of the experimental mice was assessed using a set of tests, 1 and 24 h following the injury. The tests included hind–leg flexion reflex (when raised by the tail), righting reflex (of falling on all four legs after a short drop), corneal reflex (blinking response), secretory signs (around the mouth and the eye), strength, beam balance task, beam-walking coordination task, and exploration and locomotor activity tests, as described previously (Zohar et al. 2003).

Novel Object Recognition Paradigm

An object recognition task was used to appraise recognition memory (Dix and Aggleton 1999). This task takes advantage of a propensity of rodents to discriminate a familiar from a new object. Mice were initially individually habituated to an open field box ($59\times59\times20$ cm) for 5 min, 24 h before the test. During the acquisition phase, two objects (A and B) of identical material, which were sufficiently heavy and high to ensure that mice could neither move nor climb over them, were placed in a symmetric position within the chamber for 5-min duration. At 24 h after acquisition phase training, one of these objects (A or B randomly) was substituted by a novel one (C), and exploratory behavior was again evaluated for 5 min (discrimination phase). All objects were thoroughly cleansed (70 % ethanol) between sessions to preclude odor

recognition. Exploration of an object was characterized as rearing on it or sniffing it at a distance of less than 2 cm and/or touching it with the nose. Successful recognition was revealed by preferential exploration of the novel object. Discrimination of visual novelty was assessed by a preference index (Dix and Aggleton 1999), determined as: (time near the new-time near the old object)/(time near the new+time near the old object). Pharmacological treatments were evaluated during the novel object recognition test (ORT). These included the effects of thrombin, PAR-1 agonist, PAR-1 antagonist, and saline controls injected 1 h after the acquisition phase according to ICV procedure described earlier in this section. In another series of experiments mTBI groups were subjected to the head trauma 1 h following the acquisition phase. Immediately following the trauma, some of the animals were injected ICV with normal saline or 1 μM PAR-1 antagonist.

Electrophysiological Assay

Slice and electrophysiological recordings were performed as previously described (Maggio and Segal 2012). Briefly, mice were rapidly decapitated, the hippocampus was removed, and 400 μm slices were prepared using a vibroslicer. Slices were then incubated for 1.5 h in a humidified, carbogenated (5 % CO_2 and 95 % O_2) gas atmosphere at 33±1 °C and were perfused with ACSF [containing (in mm) 124 NaCl, 2 KCl, 26 $NaHCO_3$, 1.24 KH_2PO_4, 2.5 $CaCl_2$, 2 $MgSO_4$, and 10 glucose, pH 7.4] in a standard interface chamber. Recordings were made with a glass pipette containing 0.75 m NaCl (4 MΩ) placed in the stratum radiatum CA1. Stimulation was evoked using a Master 8 pulse stimulator (A.M.P.I., Jerusalem, Israel) and was delivered through a set of bipolar nichrome electrode. LTP was induced by high-frequency stimulation consisting of 100 pulses at twice the test intensity, delivered at a frequency of 100 Hz (100 Hz, 1 s). Before applying the tetanic stimulation, baseline values were recorded at a frequency of 0.033 Hz. Responses were digitized at 5 kHz and stored on a computer. Offline analysis and data acquisition were performed using the Spike 2 software (Cambridge Electronic Design, UK). All numerical data are expressed as mean±standard error of mean (SEM), and EPSP slope changes after tetanic stimulation were calculated with respect to baseline. There were no systematic differences in the magnitudes of the baseline responses in the different conditions. All values reported refer to 30 min after tetanic stimulation. Unless otherwise indicated, statistical evaluation was performed by either a Student's *t* test for paired or unpaired data or a one-way analysis of variance (ANOVA), as the case may be (Origin 6.0). *p* Values of <0.05 were considered a significant difference between means.

Statistical Analysis

Results are expressed as the mean values±SEM. Statistical analysis for the results of the assays was carried out using *t* tests. Statistical analysis for behavioral tests was carried out using multivariate one-way ANOVA or *t* test. All analyses were performed by SPSS software (Chicago, IL, USA). One asterisk in the figures represents $p < 0.05$, two asterisks represent $p < 0.005$. All comparisons are made to sham animals unless stated otherwise.

Results

Mice were subjected to the extensive neurological assessment described in the "Material and Methods" section and showed no neurological deficits 1 and 24 h after the injury (data not shown). Thrombin activity in brain slices rose immediately following the trauma as can be seen at the 5 min post-mTBI time point in Fig. 1a. Such an elevation was expected since the putative micro-hemorhages and BBB disruption would be most prominent immediately following the trauma. Thrombin-like activity returned to its baseline similar to sham-treated animals 1 h following the trauma. Addition of the specific thrombin inhibitor (NAPAP) to the reaction mixture lowered all the detected activity levels to the baseline similar to sham animals, indicating that most of the mTBI induced activity is indeed thrombin specific.

The elevated levels of thrombin generated during the acute mTBI would be expected to cleave PAR-1 and lead to its degradation or internalization (Luo et al. 2007). As seen in Fig. 1b, PAR-1 levels measured by immunoblot displayed a trend to decrease in the first post-traumatic time points (5 min, 1 h).

In order to assess whether the brief elevation of thrombin activity immediately following mTBI could mediate amnesia, we tested the effects of ICV injection of thrombin on learning and memory in the ORT. The injection of thrombin or other control solutions was performed 1 h after the acquisition phase of the task and the animals were tested 23 h later. As can be seen in Fig. 2, saline-injected animals had excellent recall with a mean preference index of over 0.3, indicating that they spent a significantly greater proportion of time with the new object. In contrast, animal injected with thrombin spent equal time with both objects indicating no recall of the object presented to them 24 h previously ($p < 0.05$ compared to the saline-injected animals, *t* test). Injection of a PAR-1 agonist peptide had a similar deleterious effect on memory ($p < 0.05$ compared to the saline-injected animals) while co-injecting a PAR-1 antagonist completely blocked the effects of thrombin and the PAR-1 agonist.

The putative effect of thrombin and PAR-1 in causing amnesia in mTBI was confirmed by performing the ORT on

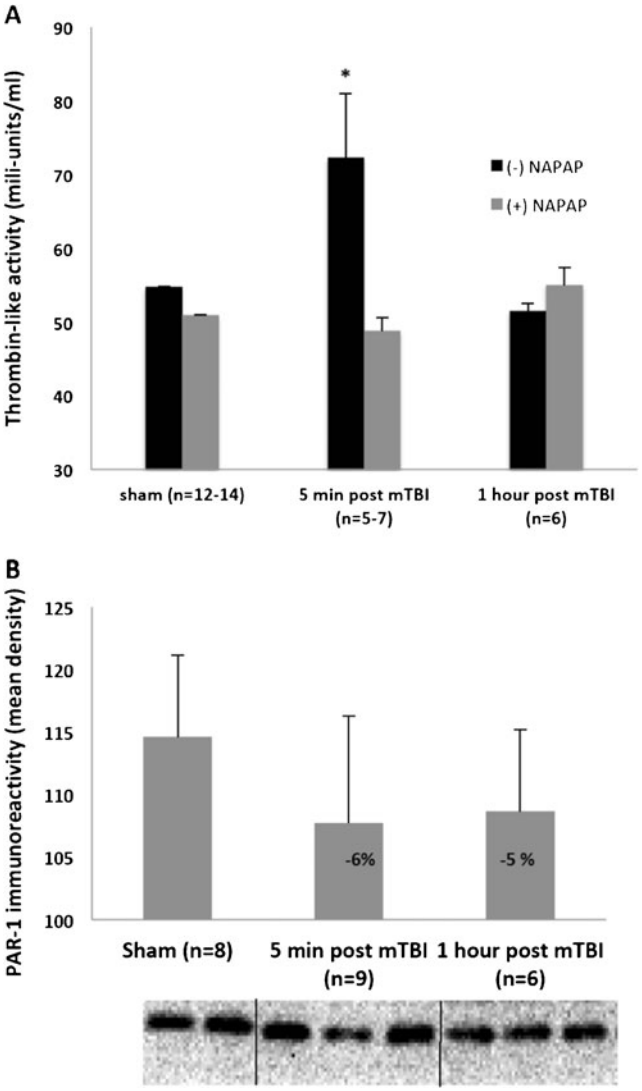

Fig. 1 **a** Thrombin like activity elevation in brain slices. Thrombin like activity (black) is significantly elevated (32 %, $p<0.05$) 5 min following the trauma and returns to control baseline in the subsequent hour. Addition of the specific thrombin inhibitor NAPAP to a reaction mixture (*grey*) reduces the levels of thrombin like activity to a baseline in all groups. Thrombin activity units were calculated using a calibration curve as described in the methods. **b** Western blot analysis of PAR-1 immunoreactivity in brain homogenates. Densitometry of PAR-1 reactive bands (representative image) reveals a trend to decrease 5 min and 1 h following the trauma, suggesting putative PAR-1 activation

the animal mTBI model. As can be seen in Fig. 2, mTBI performed 1 h after the acquisition phase of the test had exactly the same amnestic effect as thrombin injected at this point. Treatment of the animals 10 min after the trauma with saline had no beneficial effect, while treatment with the specific PAR-1 antagonist SCH completely abolished the amnestic effects of the trauma resulting in normal performance of the mTBI animals in discrimination phase of the test performed 24 h later ($p<0.005$ compared to the mTBI- and saline-treated mTBI animals).

In order to replicate the cognitive and biochemical findings, electrophyiological studies were carried out at similar time points and with similar exposure to mTBI and to thrombin. In electrophysiological tests performed 1 day following mTBI, we tested the ability of hippocampal slices from mTBI-exposed animals to undergo LTP (Fig. 3a). This figure represents the electrophysiological characteristics during the discrimination phase of the ORT performed 24 h after either mTBI or thrombin ICV injection. Notably, following tetanic stimulation, a lower LTP could be evoked in slices from mTBI-exposed mice compared to sham control animals. Indeed, post-tetanic potentiation (PTP) was 1.46±0.041 compared to 1.80±0.039 ($n=6$ animals/group; $p<0.001$), while at 55 min following potentiation, values were 1.36±0.043 and 1.77±0.034, respectively ($p<0.01$). In order to evaluate whether a PAR1 block might restore LTP in mTBI-exposed animals, we evoked LTP in slices from such animals that also received ICV injections of 1 μM PAR-1 antagonist SCH. Interestingly, SCH completely reversed the effect of mTBI on LTP. PTP was 1.79±0.023 in mTBI animals treated with SCH compared to 1.49±0.038 in control animals which received a saline injection following mTBI. A similar effect was detected at a later stage of potentiation (at 55 min. following tetanus, 1.75±0.23 in TBI animals treated with SCH vs. saline-injected TBI-exposed animals, $p<0.01$). An overall ANOVA between the tested groups revealed a significant difference among all groups of animals ($F=9.62$, $p<0.001$ with a post hoc Tukey test that detected a significant difference ($p<0.01$) among TBI vs. TBI+SCH animals, TBI+saline vs. TBI+SCH, control vs. TBI and control vs. TBI+saline).

We also measured LTP in mouse hippocampal slices 24 h after ICV thrombin injection (Fig. 3b). Interestingly, PTP as well as later stages of potentiation were also impaired in these animals [PTP: 1.53±0.038 in thrombin-injected animals vs. 1.81±0.036 in sham, control animals ($p<0.01$) and LTP: 1.27±0.033 vs. 1.75±0.033, respectively]. Notably, ICV injection of the PAR1 agonist mimicked the effects of thrombin on LTP, and in contrast, co-ICV injections of thrombin and SCH abolished the outcomes of thrombin on LTP. An overall ANOVA between the tested groups revealed a significant difference among all groups of animals ($F=6.40$, $p<0.001$ with a post hoc Tukey test that detected a significant difference ($p<0.01$), among others, between thrombin- and SCH-injected animals as well as thrombin- and thrombin+SCH-injected animals.

Discussion

The presented results support a rise in thrombin activity following mTBI, which is directly implicated in causing synaptic

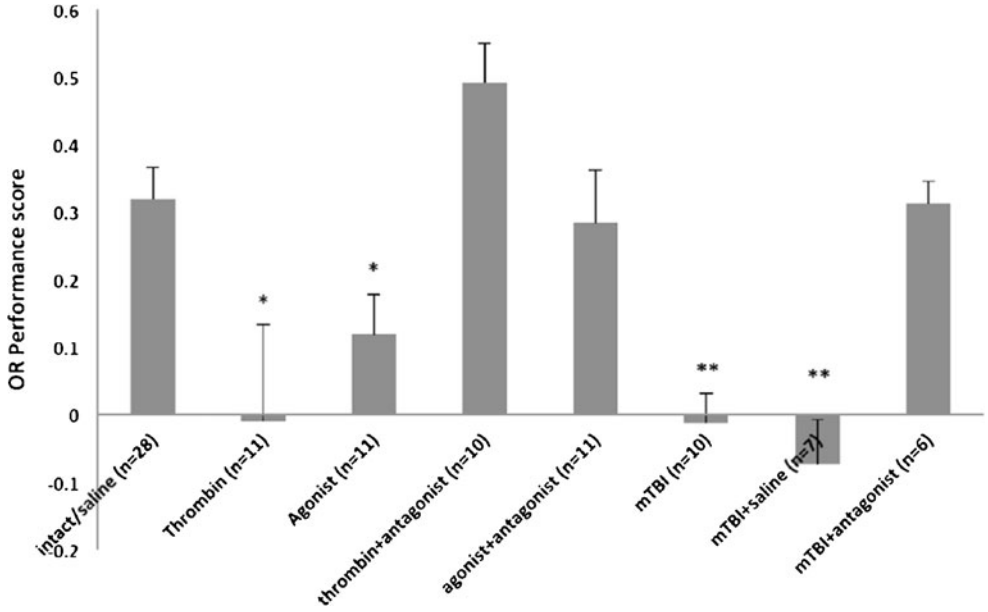

Fig. 2 Object recognition (OR) test discrimination phase was performed 1 day following ICV injection or mTBI. Results are presented as the preference index with standard error bars calculated as described in the "Materials and Methods" section. The groups of mice included those injected ICV with thrombin, PAR-1 agonist, thrombin with the PAR-1 antagonist SCH79797 and the PAR-1 agonist and antagonist together and groups of mice after mTBI, and mice-injected ICV with saline or the PAR-1 antagonist immediately after induction of mTBI. mTBI, similarly to thrombin or PAR-1 agonist injections significantly impaired animals ORT performance. Animals' performance was restored by ICV injection of PAR-1 antagonist immediately following mTBI or when administered together with thrombin or the PAR-1 agonist (*$p<0.05$; **$p<0.005$)

dysfunction and amnesia by activation of PAR-1. mTBI in the mouse model was associated with an early transient rise in thrombin activity. A brief rise in thrombin levels as an immediate effect of mTBI is expected since trauma injures blood vessels resulting in tiny bleeds. Human studies utilizing MRI techniques clearly support the existence of micro-bleeds in mTBI as reviewed recently (Benson et al. 2012).

Thrombin and related proteases may also activate PAR-4 and possibly PAR-3 in the brain. The present results support a relatively specific role for PAR-1 in generating the amnesia and electrophysiological deficits described here following mTBI since specific blockade of PAR-1 was sufficient to completely reverse the effects of both mTBI and thrombin. The trend toward lower levels of PAR-1 immediately following mTBI also supports activation of the receptor since cleavage of the receptor by thrombin both activates it and induces its internalization and degradation (Luo et al. 2007). Similarly, although trauma is expected to expose the brain to a number of coagulation factors, the excessive thrombin-like activity generated immediately following mTBI was measured in a substrate assay previously shown to be specific for thrombin (Beilin et al. 2001; Shavit et al. 2011) and was completely

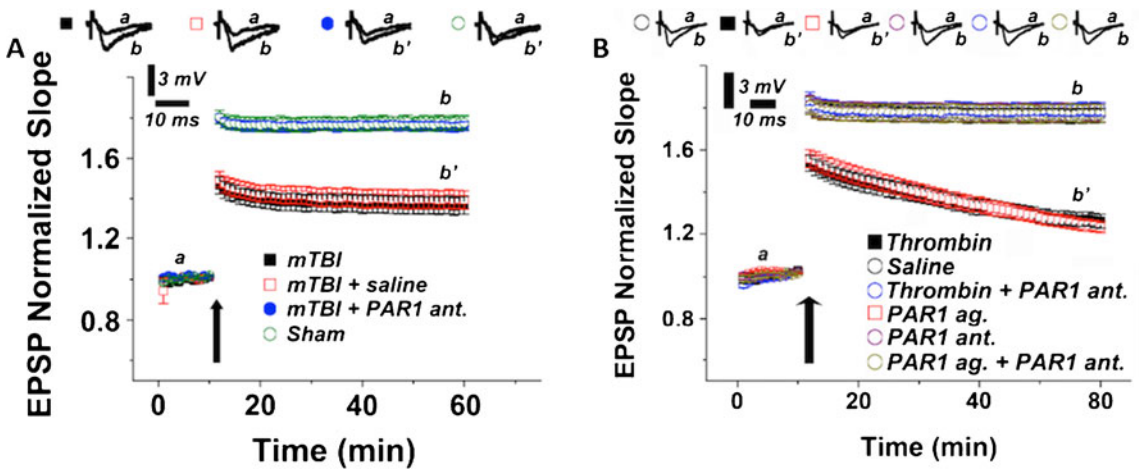

Fig. 3 **a** mTBI decreases LTP through PAR-1. Sample illustrations are at the indicated time points marked on the grouped data, which are averages of 12 slices each. **b** Thrombin ICV injections depresses LTP in hippocampus. The PAR-1 antagonist rescues the effect. Sample illustrations are at the indicated time points marked on the grouped data, which are averages of twelve slices each

inhibited by NAPAP, which is a highly specific thrombin inhibitor at the concentrations used. It is important to note that thrombin is the final enzyme in the coagulation cascade and it is likely that both its high activity and specificity for PAR-1 relative to other coagulation factors is the main activator of PAR-1. These considerations indicate therefore that thrombin acting through PAR-1 is the major factor in causing synaptic dysfunction and memory loss after brain trauma.

The electrophysiological studies were performed at the same time point at which the discrimination phase of the ORT was performed, 24 h after challenging the brain with either mTBI or ICV injection of thrombin. These results confirm that PTP is significantly impaired at this point, indicating again that a relatively brief exposure to elevated levels of thrombin has the capacity of long-term impairment of synaptic function. The reversal of these electrophysiological effects by the prompt administration of a PAR-1 antagonist, together with thrombin or immediately after mTBI, strongly suggests that the deleterious effects are mediated through this receptor. The cognitive and pharmacological data presented clearly support a link between the elevation in thrombin activity with the amnesia associated with mTBI: Both mTBI, thrombin, and a PAR-1 receptor agonist induced amnesia to information acquired 1 h previously, and the amnestic effects of all these could be blocked by ICV administration of the a specific PAR-1 antagonist. The type of amnesia displayed in the current test involves loss of memories acquired 1 h previously and seems to best fit retrograde amnesia, which is the hallmark of mTBI. The molecular basis of such an effect is hypothesized to affect the consolidation of memory. LTP impairment was originally described by us about 1 h after exposure to thrombin (Maggio et al. 2008) and was also found after the slice was no longer exposed to thrombin. Slices as used in the previous experiment cannot be maintained for 24 h and the present results which are based on the exposure of the brain in vivo indicate that this effect of thrombin on LTP persists for at least 24 h. It is therefore reasonable to argue the amnesia induced by a short-term exposure to thrombin could also explain the anterograde amnesia associated with mTBI (Zohar et al. 2011).

The molecular events that follow PAR-1 activation and seem to persist for at least 24 h are likely to be complex and involve a number of putative pathways. Downstream effects of PAR-1 include Ras and ERK (Aronovich et al. 2005) activation. These effects are probably occurring also in astrocytes and especially on the astrocyte endfeet located at the synapse (Lee et al. 2007; Shavit et al. 2008, 2011). It is possible that signal pathways in astrocytes mediated by PAR-1 cause relatively long-lasting changes in protein expression and function mediated by downstream nuclear signals. The astrogliosis seen following mTBI (Tashlykov et al. 2007, 2009; Tweedie et al. 2007) may be, in part, driven by such stimulation of PAR-1 receptors.

The present results indicate the potential for new therapeutic strategies aimed at thrombin activation of PAR-1 for alleviating amnesia and possibly other cognitive and behavioral deficits following mTBI in humans. Modulating this pathway may also have a beneficial effect on the long-term consequences of mTBI, and future studies should be aimed at examining this possibility. It is important to note, however, that this approach poses potential risks since thrombin inhibition and indeed PAR-1 inhibition may complicate trauma by causing excessive bleeding in the brain. Ways of dealing with such problems should be thought out and may be relevant to a number of diseases, such as stroke and inflammation, in which excessive activation of PAR-1 may play a major role.

A major finding of the present study is that immediate ICV injection of the PAR-1 antagonist SCH can reverse the short-term amnesia. It remains to be seen whether this will also have an effect if given later following mTBI. Such antagonists have been used for other purposes (Tricoci et al. 2012) in human subjects and have been associated with a risk of hemorrhage in patients with stroke. It may be possible to give low doses of these medications in patients with mTBI. This question would require a large series of experiments outside the scope of the present study. Clinically, the present study indicates that the treatment of mTBI with a PAR-1 antagonist up to 1 h following trauma would be potentially effective in ameliorating amnesia. This is a novel and modifiable therapeutic target and may be clinically relevant to the acute treatment of mTBI. It is important to note that the effects of PAR-1 over activation are early and irreversible. Very short and focused antagonism of PAR-1 may be effective and not associated with excess bleeding complications.

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Conflict of interest None.

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